

Ettan Spot Handling Workstation

System Manual
Ettan Spot Handling Workstation 2.1



Important user information

All users must read this entire manual to fully understand the safe use of Ettan Spot Handling Workstation.

WARNING!



The WARNING! sign highlights instructions that must be followed to avoid personal injury. It is important not to proceed until all stated conditions are met and clearly understood.



WARNING! Avoid exposure to hazardous laser radiation.

CAUTION!

The Caution! sign highlights instructions that must be followed to avoid damage to the product or other equipment. It is important not to proceed until all stated conditions are met and clearly understood.

Note

The Note sign is used to indicate information important for trouble-free and optimal use of the product.

CE Certifying

This product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

The **CE** symbol and corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked GE Healthcare instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from GE Healthcare except for alterations described in this manual.

Recycling



This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of your equipment.

WARNING!

This is a Class A product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

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1 Introduction

IMPORTANT! Ettan™ Spot Handling Workstation is intended for research use only and should not be used in any clinical procedures for diagnostic purposes.

Ettan Spot Handling Workstation is a fully integrated instrument for automatic processing and transfer of biomolecules from polyacrylamide electrophoresis gels to targets used in MALDI-ToF mass spectrometer (MS). Processing is done in three steps:

1 **Spot picking** from 2-D electrophoresis gels.

This step is defined as removing material of interest from polyacrylamide electrophoresis gels (contained in gel plugs) and dispensing it into microplate wells.

2 **Digestion** of the picked proteins.

This step is defined as pre-treatment (washing, destaining, etc.), enzymatic cleaving and extracting biomolecules from the gel plugs. Digestion comprises a number of substeps, including dispensing into microplate wells and drying, both as an intermediate and a last step.

3 **Spotting** (dispensing) of samples onto targets for the MS.

This step is defined as pre-treatment (dissolving, etc.) and transfer of extracted biomolecules and matrix solution from microplate wells or containers to MALDI targets.

1.1 *The System Manual*

This manual provides technical information, process descriptions and start-up/shutdown instructions for Ettan Spot Handling Workstation. In addition, maintenance schedules and instructions for user maintenance are included.

1.2 *System overview*

Ettan Spot Handling Workstation consists of the following sub-systems (see Fig. 1-1):

- Cabinet
- Spot Picker & Spotter
- Digester with dryer and incubator
- Transfer robot
- Gel hotel
- Microplate/MS sample tray hotel
- Ettan Spot Handling Workstation control software running under Windows 2000® operating system on a PC
- PC with frame grabber card for video display

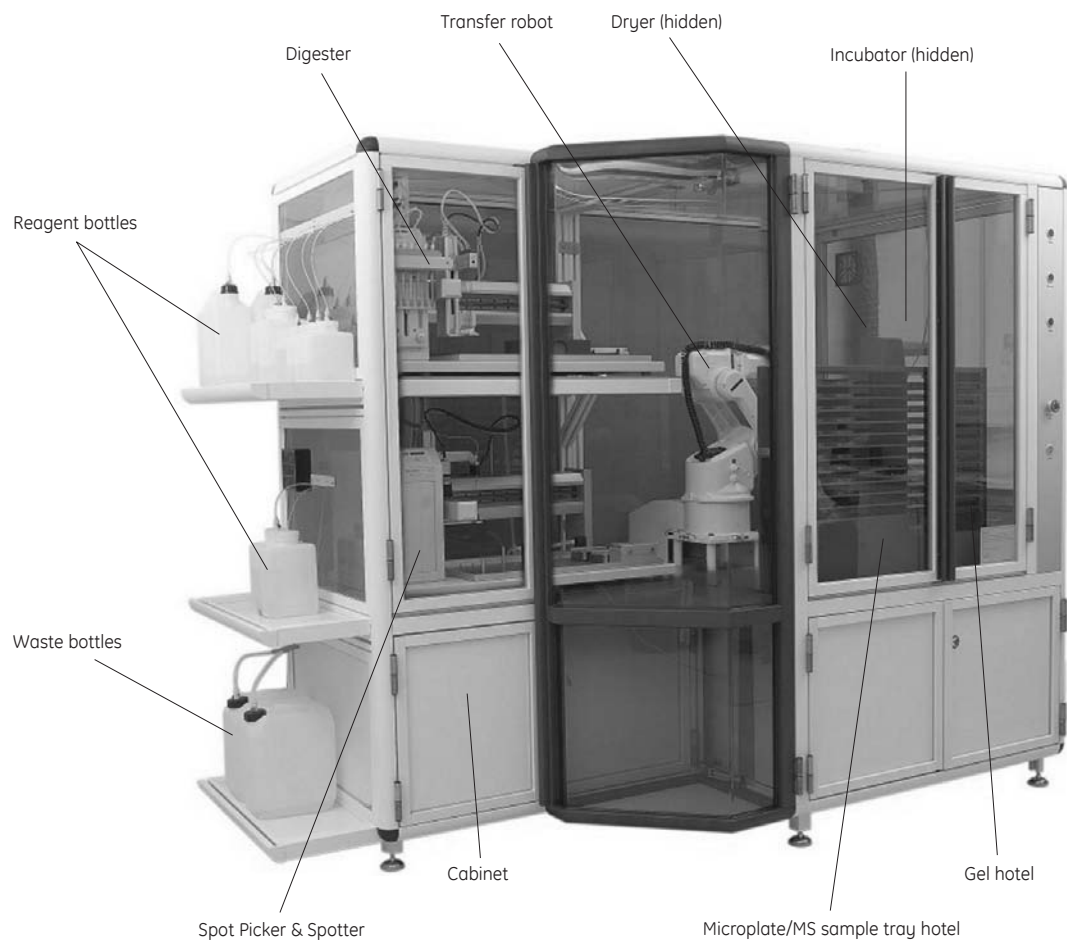


Fig 1-1. Ettan Spot Handling Workstation.

1 Introduction

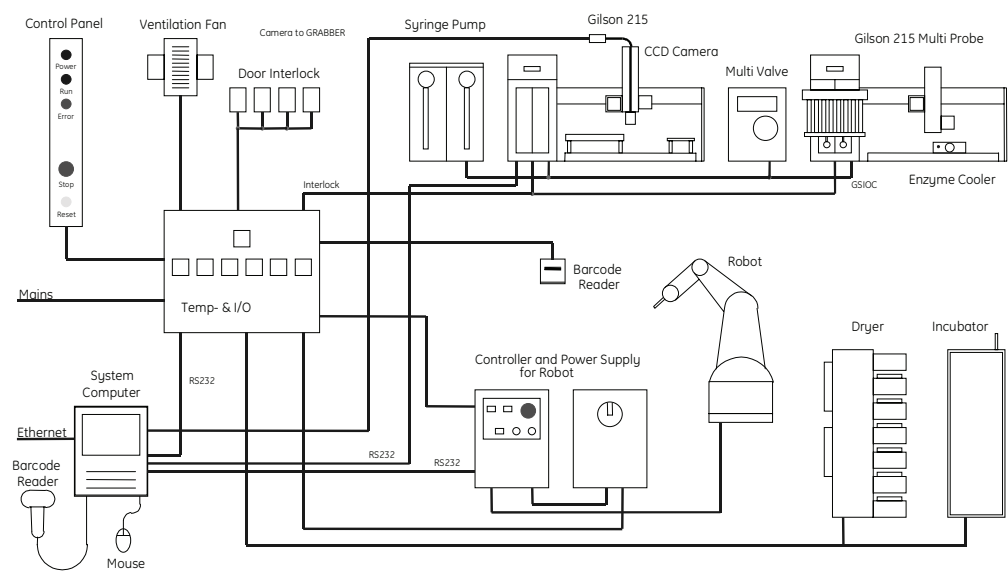


Fig 1-2. Ettan Spot Handling Workstation configuration.

The sub-systems are described in detail in the System description chapter of this manual, where brief descriptions of the individual components are also given. Ettan Spot Handling Workstation control software is described in the separate Ettan Spot Handling Workstation User Manual and Online Help.

The package contents are described in Section 1.3.

1.2.1 Rating label

The rating label is located at the right side of cabinet close to the mains connections. The layout of rating label, see figure below.

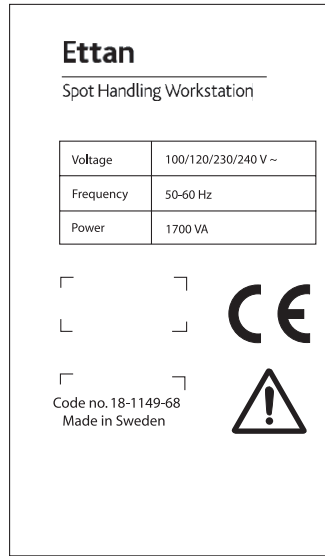


Fig 1-3. Rating label layout.

1.3 Package contents

Ettan Spot Handling Workstation is delivered with the products listed in Table 1-1. For ordering information, refer to Section 6.4.

Table 1-1. Ettan Spot Handling Workstation, delivered products.

Product	Quantity
Ettan Spot Handling Workstation control software	1
Picker head 1.4 mm for 1.0 mm thick gels	2
Picker head 2.0 mm for 1.0 mm thick gels	2
Reference markers, sheet incl. 560 pieces	2
Gel tray	12
Gel holder	24
Glass lid for gel tray	12
Tubing, Spot Picker & Spotter	1
Tubing, Digester	1
Tubing, waste	1
Camera calibration foot	5
Calibration plate	1
Waste bottle, 15 liter	2
Reagent bottle, 5 liter	3
Reagent bottle, 2 liter	3
Reagent bottle, 1 liter	4

1.4 Accessories

In addition to the items delivered with the system, Ettan Spot Handling Workstation requires the following accessories:

- Image scanner: ImageScanner™, or Typhoon™*
- Gel image evaluation software, e.g. ImageMaster™ (Nonlinear Dynamics)

See Chapter 6 for specifications and ordering information.

* If you want to use other scanners, please contact GE Healthcare.

1.5 Associated documentation

1.5.1 Ettan Spot Handling Workstation Safety Handbook

Provides the safety instructions that must be strictly followed for the safe use of Ettan Spot Handling Workstation.

1.5.2 Ettan Spot Handling Workstation Site Preparation Guide

Provides the data needed to prepare the laboratory for the installation of Ettan Spot Handling Workstation.

1.5.3 Ettan Spot Handling Workstation User Manual

Contains detailed operating instructions.

1.5.4 Ettan Spot Handling Workstation Online Help

An online help function can be reached from the software. A troubleshooting guide is also included in this function.

1 Introduction

2 System description

2.1 General



Fig 2-1. Ettan Spot Handling Workstation.

Ettan Spot Handling Workstation consists of fully integrated instruments for the automatic processing and transfer of biomolecules from polyacrylamide electrophoresis gels to MALDI-ToF MS targets. To control the system, a personal computer running Ettan Spot Handling Workstation control software is included.

Communication between the computer and the various modules and components of Ettan Spot Handling Workstation is achieved via high speed data network connections.

Reagent bottles for the fluid handling equipment and the syringe pump module are placed on shelves to the left of the cabinet. The waste bottles are placed on the bottom shelf. The bottles are connected to the instruments via tubing through the cabinet wall, see Fig. 2-2.

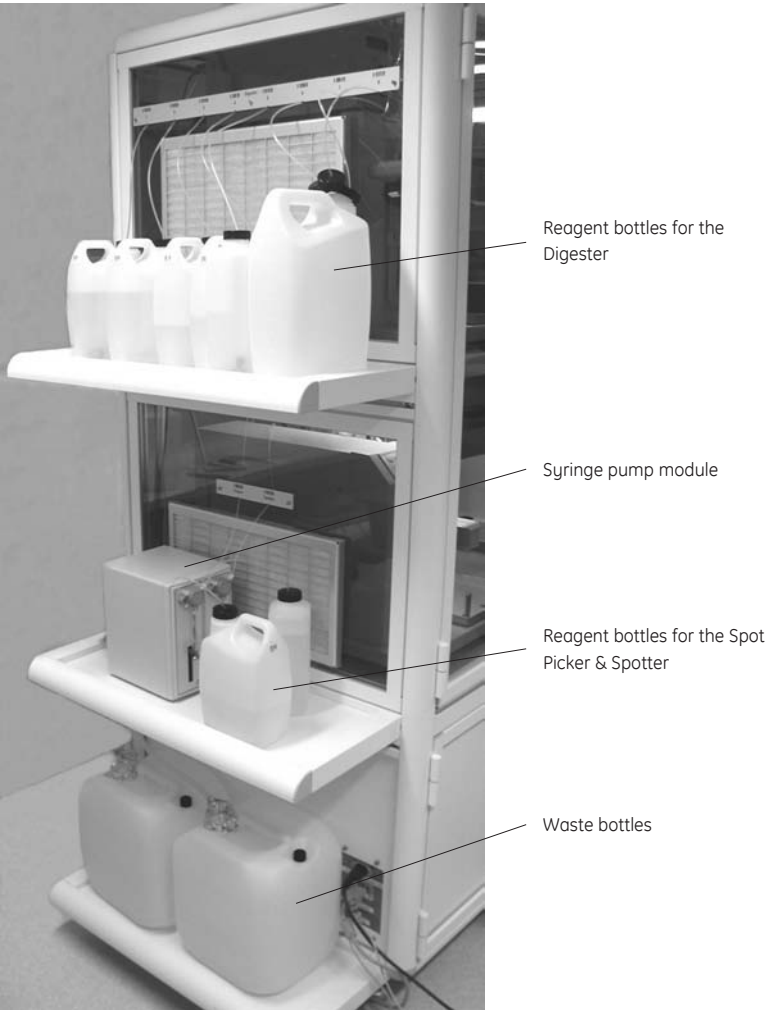


Fig 2-2. Reagent/waste bottles and syringe pump module.

2.2 The Cabinet

The purpose of the cabinet is to house all mechanics, for operator and personnel safety, to protect personnel from hazardous chemicals, and to avoid contamination of samples in process. The operator has access to all components via the cabinet doors.

The cabinet consists of two main parts:

- 1 The left part contains (see Fig. 2-3):
 - Digester and Spot Picker & Spotter (upper compartment)
 - computer and temperature-I/O controller (lower compartment)
- 2 The right part contains (see Fig. 2-4):
 - robot, dryer, incubator and hotel for gel trays, microplates and MS sample trays (upper compartment)
 - power supply and control equipment for the robot (placed on a sliding shelf in the lower compartment)

The cabinet is equipped with an evacuation fan for transporting process air to the ventilation system. Since most of the evacuated air will contain fumes that are heavier than air, the inlet to the evacuation fan is located at a low level. In addition, the cabinet has filters for the incoming air to minimize contaminants in the cabinet.

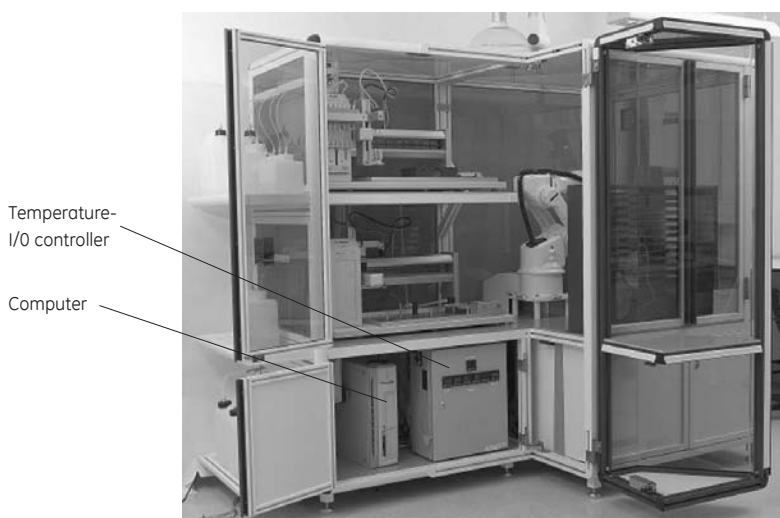


Fig 2-3. The cabinet's lower left compartment.

2 System description



Fig 2-4. The cabinet's lower right compartment.

2.2.1 Front panel controls and indicators

The layout of the Ettan Spot Handling Workstation front panel is shown in Fig. 2-5.

The front panel has two control buttons:

Emergency Stop When activating this red push-button, the workstation's instruments and the transfer robot immediately cease movement and all motors are de-energized.



WARNING! If the Picker/Spotter or the Digester is in its homing procedure when the emergency stop button is activated, the homing will be completed before the instrument ceases movement. Homing means that the unit is seeking its zero position. The text "HOMING" is shown in the unit's display.

Reset	This non-latching push-button is used to acknowledge closed cabinet doors. The push-button's yellow indicator is on when one or several cabinet doors are opened, and will go off after acknowledgement.
Power	This blue indicator is on when the mains power is switched on, i.e. when power is present in the workstation.
Running	This blue indicator is on during batch processing, and is flashing when a batch process is in Pause Batch mode. The indicator is off when no batch is present in the workstation.
Error	This red indicator is on if an error occurs, and is flashing when a warning appears in the Handle Events dialog. To acknowledge the warning, select the warnings row, and click Clear warning . The error indicator will stop flashing.

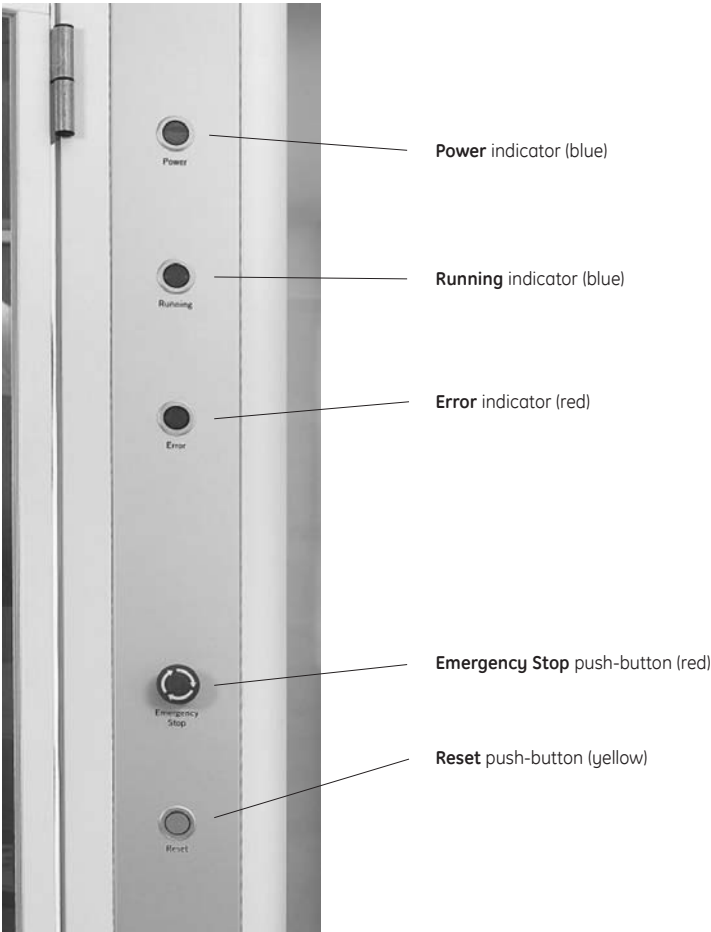


Fig 2-5. Front panel controls and indicators.

2.2.2 External controls and connections

The controls and connections located at the right side of the cabinet are shown in Fig. 2-6.

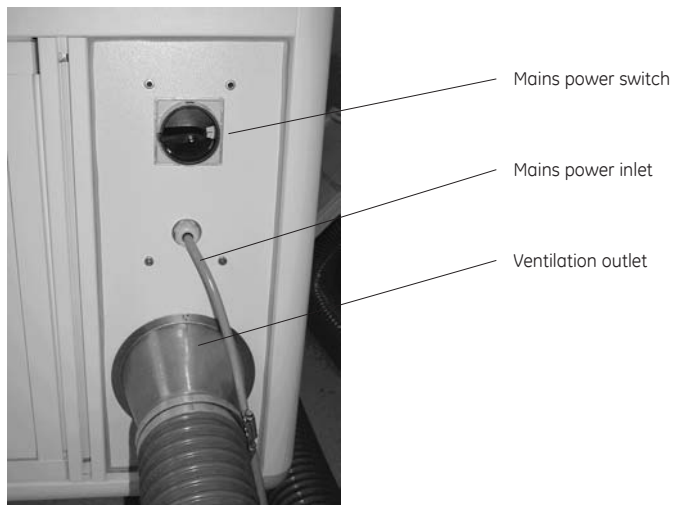


Fig 2-6. Mains connections and ventilation outlet.

The following connections are located at the left side of the cabinet, see Fig. 2-2.

- 1 Reagent bottle tubing.
- 2 Waste bottle tubing.
- 3 Syringe pump module tubing.

2.2.3 Safety switches

The upper doors of the cabinet are equipped with safety switches. The switches are mounted at the same level as the lower part of the doors.

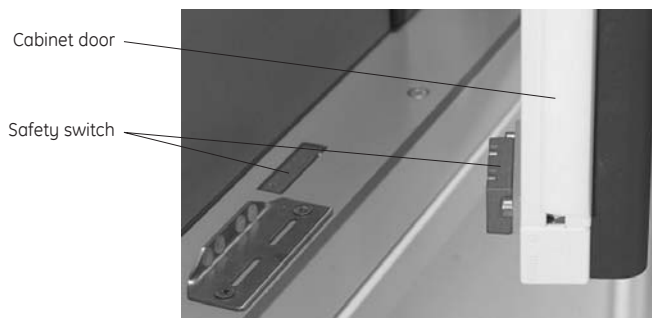


Fig 2-7. Safety switch on the cabinet door.

2.3 The Spot Picker & Spotter

The Spot Picker & Spotter is a robotic system that can move the picker/spotter head in three directions: X, Y and Z. The syringe pump (see Fig. 2-9) allows precise handling of liquid and picked gel plugs. The valve next to the syringe pump controls the flow from the liquid supply, and to/from the picker/spotter head.

The picker head and the spotter head are mounted on the same robot arm. This is possible since they are not used at the same time.

The picker head movements are guided by a camera system. The camera, which is attached to the Z-arm, is connected to the PC via a frame grabber card. Reference markers on the gel are identified using the camera. This enables the pick list coordinates from the scanned gel to be transferred to picker head positions during automated picking.

Two microplate supports and a vial holder for matrix solutions and calibrants are placed beside the gel tray.

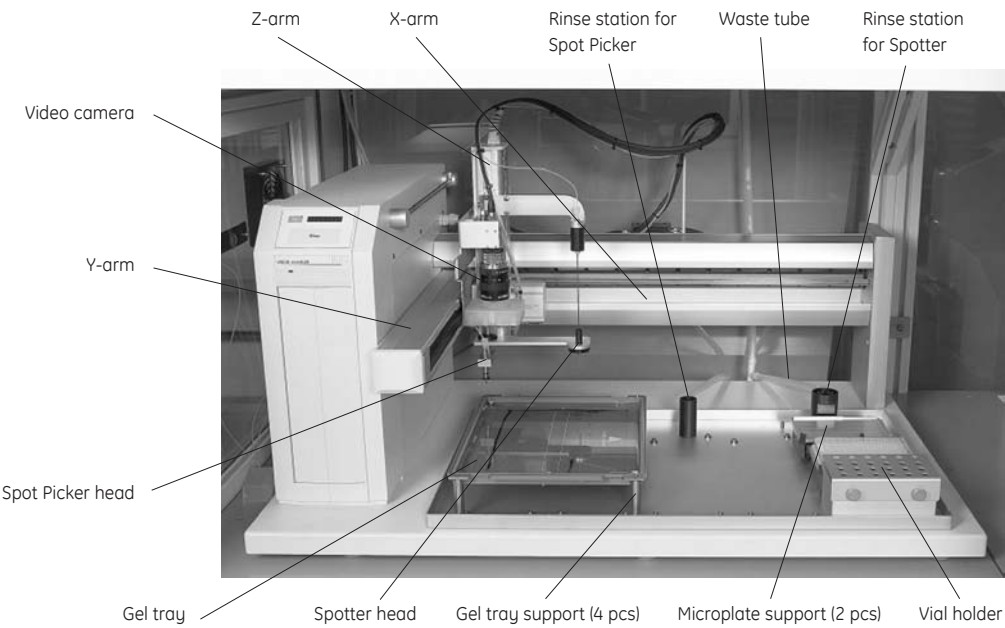


Fig 2-8. The Spot Picker & Spotter.

Note: Fig. 2-8 shows a previous spotter head design.

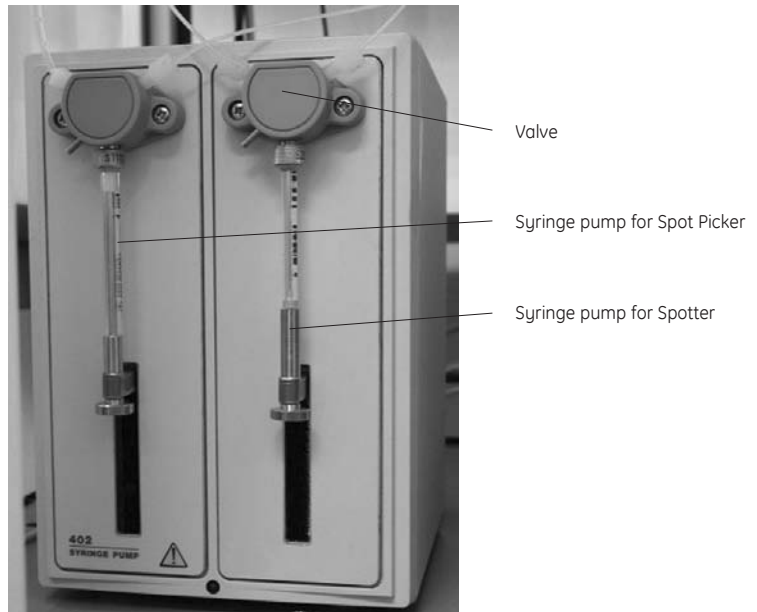


Fig 2-9. The Syringe pump module.

The syringe pump module is placed on a shelf to the left of the cabinet, see Fig. 2-2.

2.3.1 The picker head

The picker head is mounted on the Z-arm just behind the video camera.

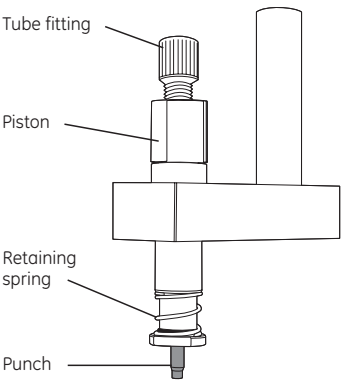


Fig 2-10. The picker head.

The retaining spring creates a pressure against the gel backing during picking, thus ensuring that the gel plug will be properly excised. When the Z-height is correctly adjusted, the picker head should be lowered until a gap is formed between the piston and the body of the picker head, thereby allowing the spring to press against the gel backing.

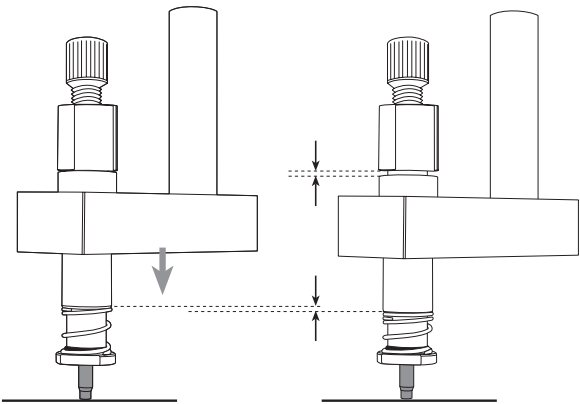


Fig 2-11. Spring action when lowering the picker head.

2.3.2 Video camera

The video camera is used to establish the coordinates for the gel to be picked. By detecting the reference markers on the gel and comparing with the coordinates in the pick list, the picking coordinates can be determined.

Since the camera lens is located at a distance from the picker head, its position relative to the picker head has to be precisely defined to ensure high precision picking. The camera alignment routine provides the means for this, see Chapter 4. Also, instructions for adjustment of camera focus and aperture are provided in Chapter 4.

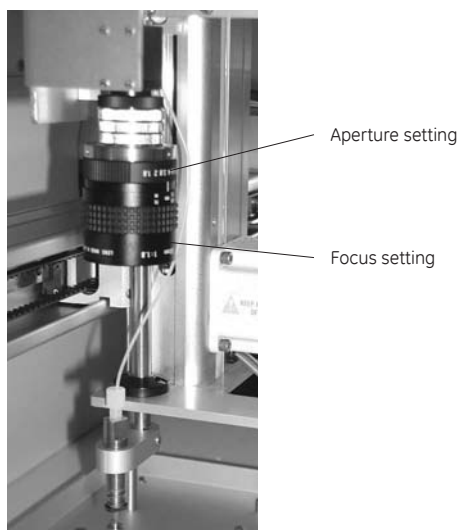


Fig 2-12. The video camera.

The video signal is processed by a frame grabber card in the PC before displaying the image in Ettan Spot Handling Workstation control software.

2.3.3 The spotter head

The spotter head is mounted on a bracket on the same Z-arm as the spot picker head.

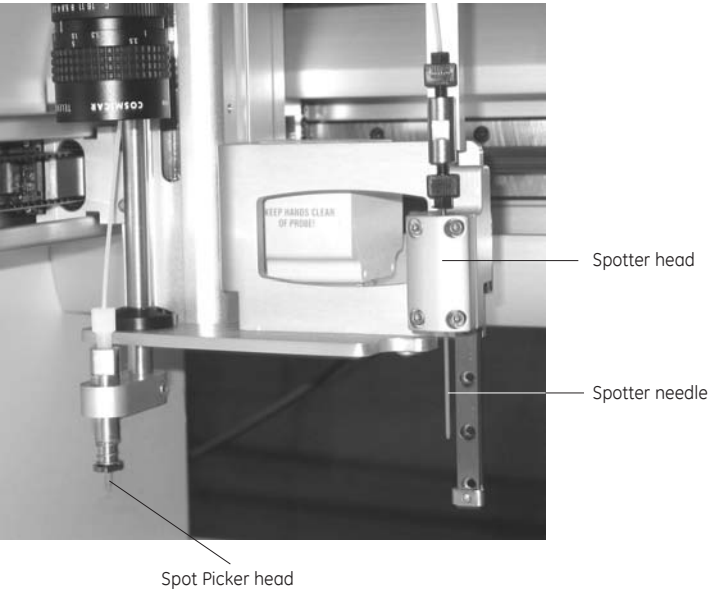


Fig 2-13. Spot picker and spotter heads.

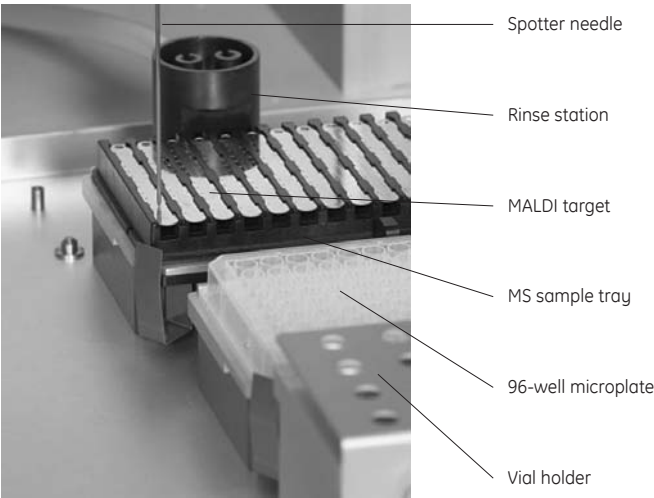


Fig 2-14. Spotting onto a MALDI target.

2.4 The digestion system

The Digester is a robotic system designed to prepare gel plugs for enzymatic digestion of proteins and subsequent extraction of peptides. The instrument has an 8-needle multiple probe head that can move in the X, Y and Z directions.

The multiple probe head is connected to an 8-channel multi-probe dilutor. An 8-position valve actuator with a common outlet is used to select the different liquid solvents.

The rinse station has two positions for rinsing; one for shallow rinsing of the needles and one for deep rinsing.

Also included in the digestion system are a dryer and an incubator, see descriptions below.

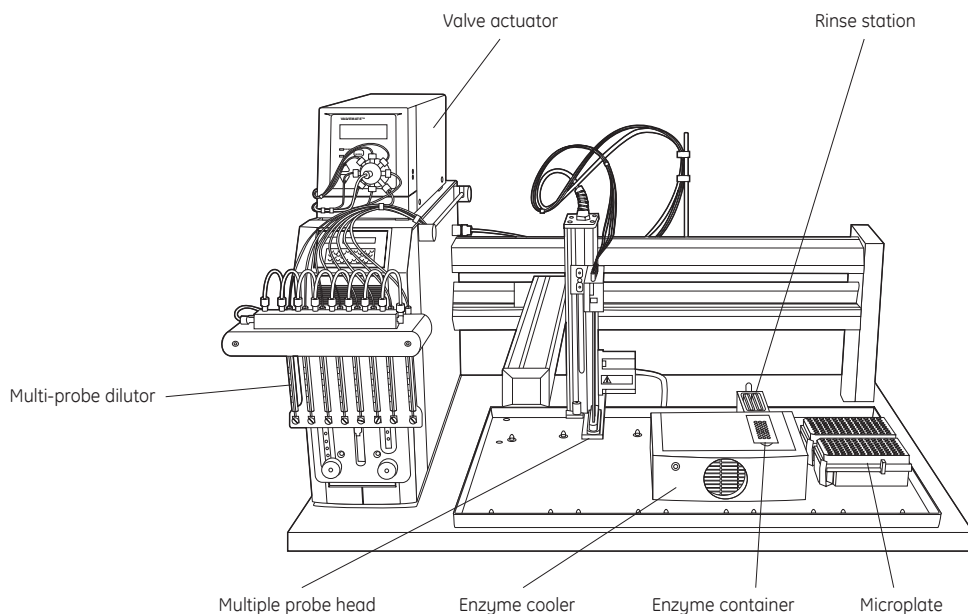


Fig 2-15. The Digester.

2.4.1 The multiple probe head

The 8-needle multiple probe head is mounted on the Z-arm.

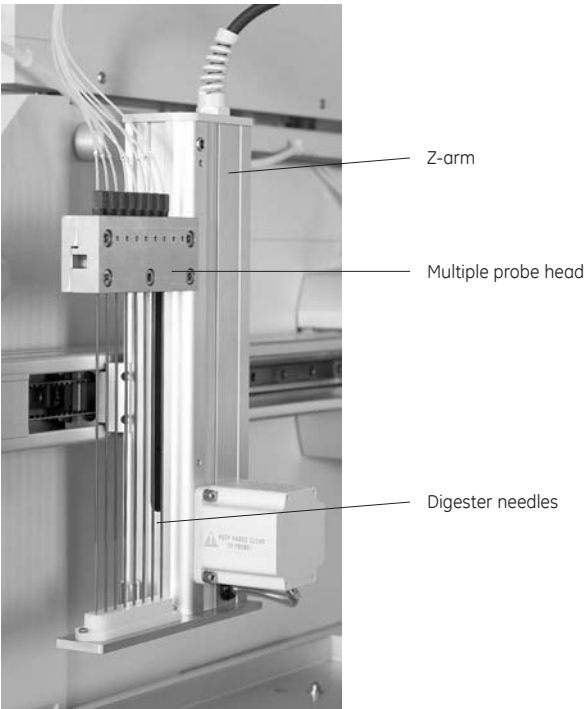


Fig 2-16. The 8-needle multiple probe head.

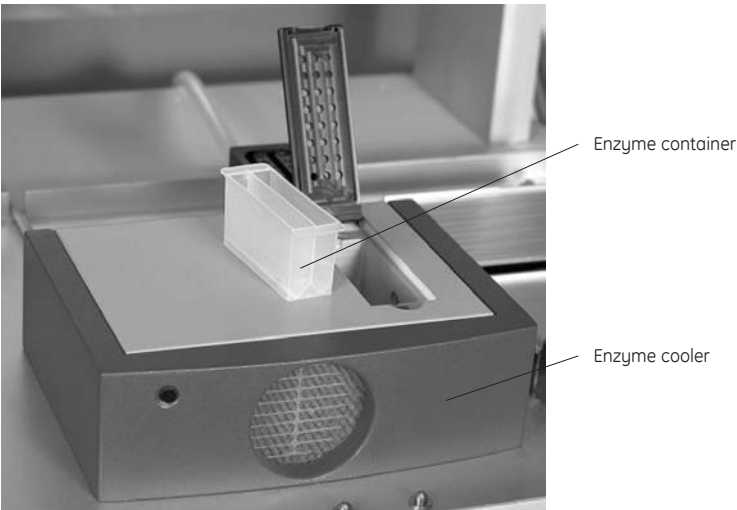


Fig 2-17. The enzyme cooler.

2.4.2 The dryer

The purpose of the dryer is to evaporate the liquid/gel plug in the microplate wells and to dry peptide extract. The dryer consists of open chambers with space for six microplates. Each microplate position has a heater located underneath.

The dryer is also equipped with fans for air circulation. An air distributor is placed above the microplate position to direct the air flow into each microplate well. An evacuation fan in the cabinet then transports the air to the ventilation system.

Note: The upper two microplate positions have no air circulation.

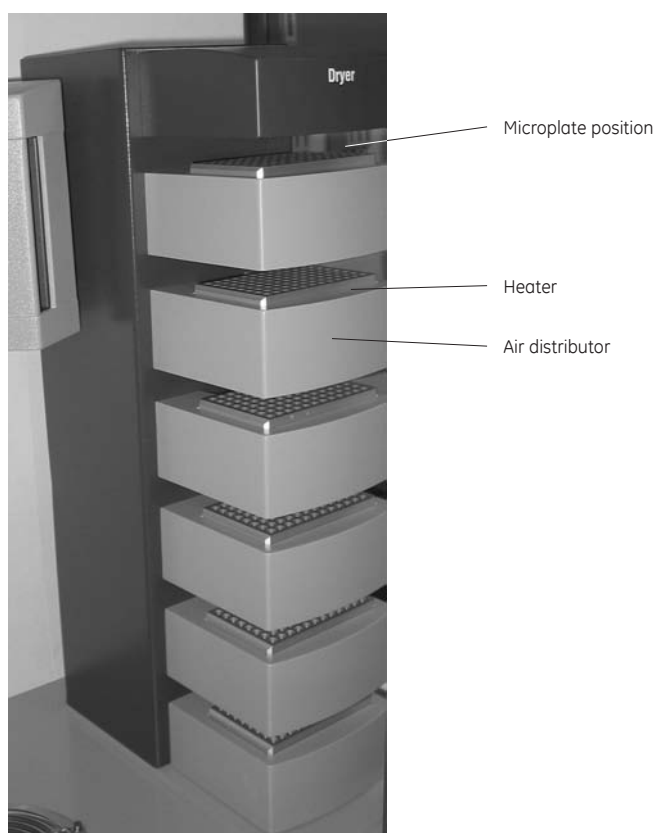


Fig 2-18. The dryer.

2.4.3 The ion generator

The purpose of the ion generator is to neutralize the electrostatic charged air surrounding the microplates when placed in the dryer, and thus minimize the electrostatic influence on the gel plugs.

The ion generator is mounted in a separate box attached to the upper part of the dryer, see figure below.

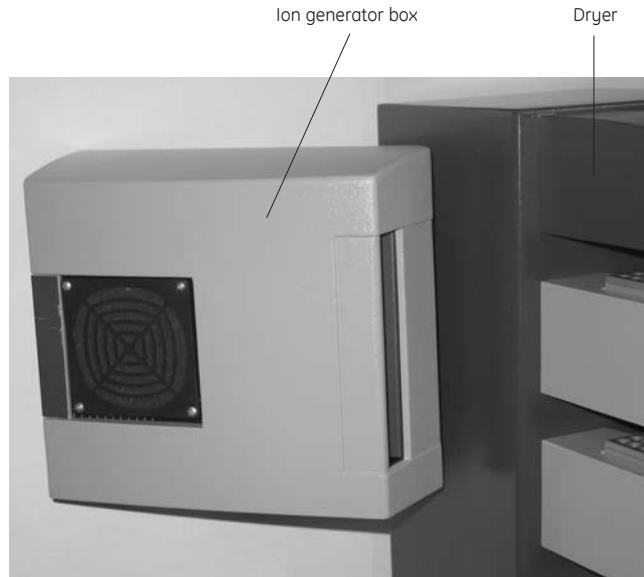


Fig 2-19. The ion generator.

2.4.4 The incubator

The purpose of the incubator is to establish a constant temperature during enzymatic digestion at a predefined time. The incubator has one closed chamber with space for twelve microplates and is equipped with heating control.

During incubation, the microplates are covered with separate lids (see Fig. 2-21) to avoid evaporation. The lid is located above each microplate position, and is placed onto the microplate by the robot when it inserts the microplate into the incubator.

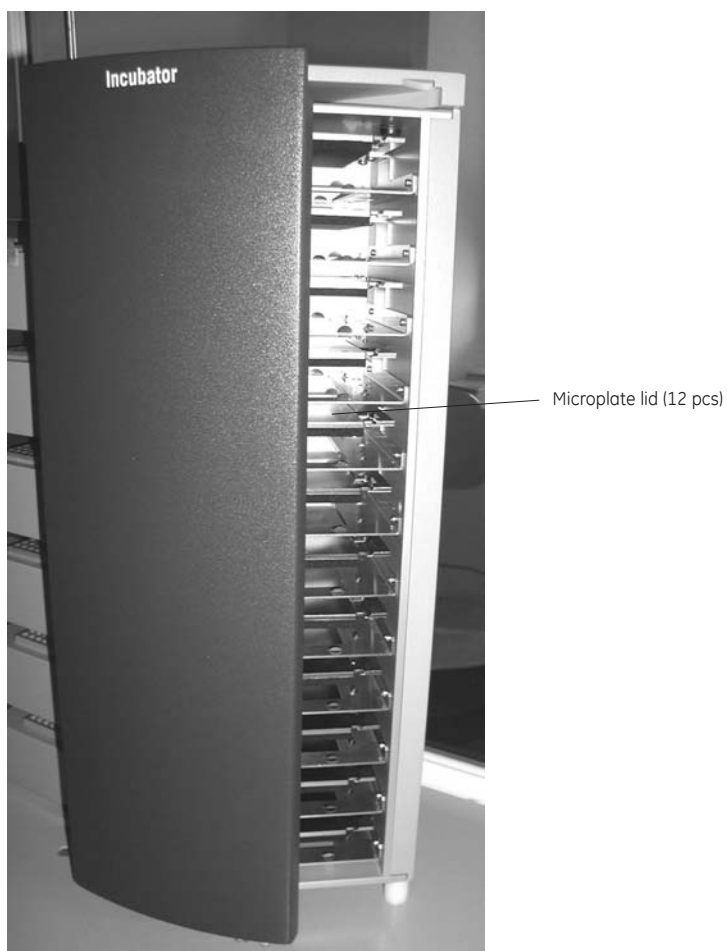


Fig 2-20. The incubator.

2 System description



Fig 2-21. Microplate lid.

2.5 The transfer robot

A robot system with six degrees of freedom is used as a bridge between the different modules in Ettan Spot Handling Workstation.

The robot handles gel trays, microplates and MS sample trays as well as opens and closes the incubator door. In addition, the robot can handle the lids for both gel trays and microplates.

The robot is equipped with a servo gripper for handling gel trays as well as microplates and MS sample trays.

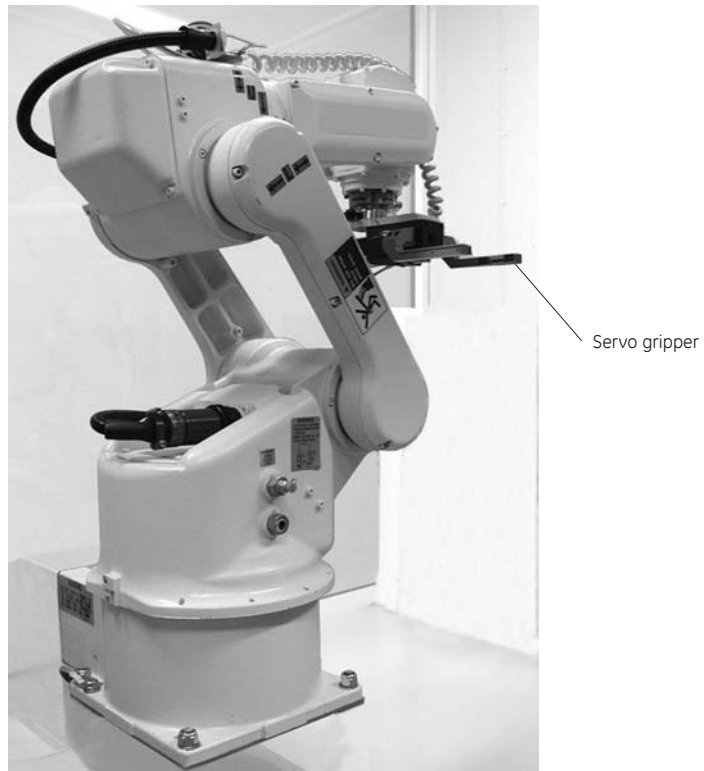


Fig 2-22. The transfer robot.

2.6 The hotel

The hotel is divided into two sections; one containing shelves for microplates and MS sample trays, and one with rails for gel trays.

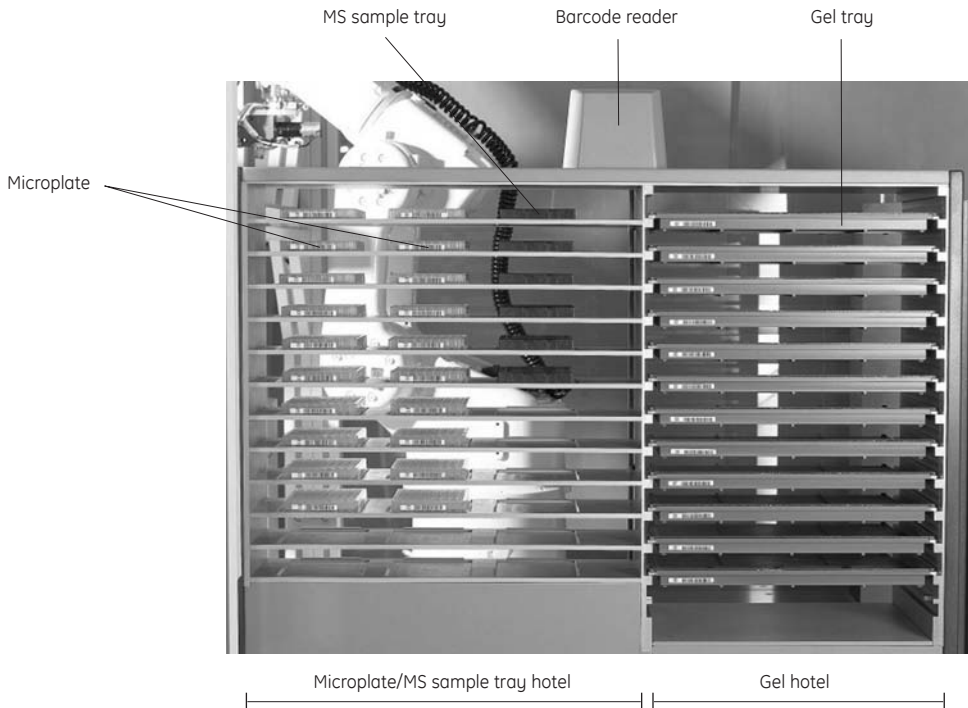


Fig 2-23. The hotel.

2.6.1 Gel hotel

The gel hotel has space for twelve gel trays. The bottom position of the hotel is used to lift off the glass lid from the gel tray. When in this position, the robot lowers the gel tray approx. 10 mm and the glass lid remains on the shelves for temporary storage.

Note: The gel tray has a silicone sealing on the top edge. This protects the gel during storage.

2.6.2 Microplate/MS sample tray hotel

The Microplate/MS sample tray hotel can house 24 microplates and 12 MS sample trays for a batch run.

2.6.3 Barcode reader



WARNING! LASER RADIATION. DO NOT STARE INTO BEAM OR VIEW DIRECTLY WITH OPTICAL INSTRUMENT. CLASS 2 LASER PRODUCT.

Do not open cover to the internal barcode reader. Refer servicing to qualified service personnel.

The barcode reader, located at the top of the hotel, is used to track samples from gel to microplate to MALDI targets. After moving a gel tray, a microplate or a MS sample tray from the hotel, the transfer robot will stop at the reading position in front of the barcode reader.



Fig 2-24. Barcode reader.

2.7 *Workflow in Ettan Spot Handling Workstation*

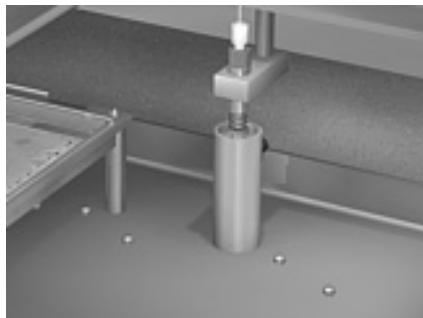
This section gives a brief step-by-step description of the workflow through Ettan Workstation. For detailed description, refer to Ettan Spot Handling Workstation User Manual.

2.7.1 **Batch run start**

- 1 The workstation's control software starts to identify all gel trays, microplates and MS sample trays.
- 2 The transfer robot moves the gel tray to the bottom position of the hotel to lift off the glass lid.
- 3 The robot moves the gel tray to the Spot Picker & Spotter.
- 4 The robot picks up a microplate from the hotel and moves it to the Spot Picker & Spotter.

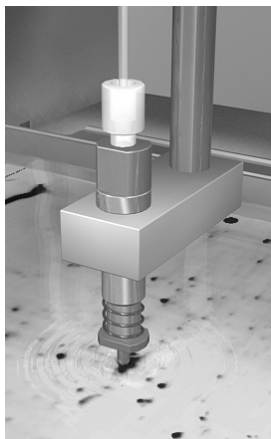
2.7.2 **Spot picking**

- 1 The Spot Picker locates the reference markers.
- 2 The picker head moves to the rinse station.

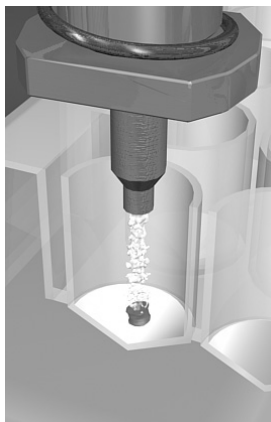


- 3 The picker head moves to a gel spot according to pick list coordinates.

- 4 The gel spot is released from the gel backing (under liquid) by punching and sideways movements ("jazz").



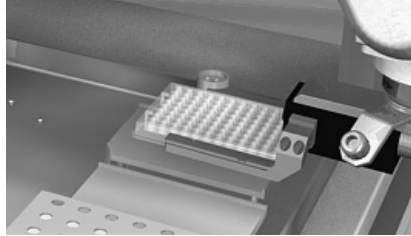
- 5 The gel plug is aspirated using Milli-Q™ water as hydraulic liquid.
- 6 The gel plug is dispensed into a microplate well together with Milli-Q water or destaining buffer.



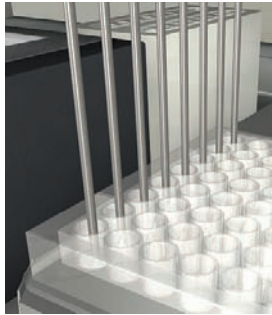
- 7 The picker head moves to the rinse station for washing using Milli-Q water (optional).

2.7.3 Digestion

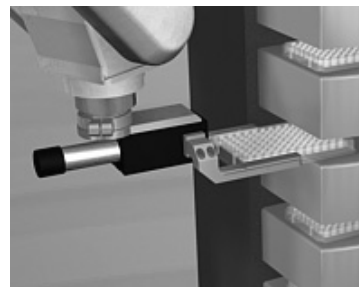
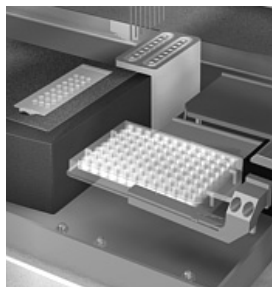
- 1 The robot moves the finished microplate with gel plugs and picker liquid up to the Digester.



- 2 The Digester aspirates the picker liquid from the gel plug wells and moves it to the rinse station.

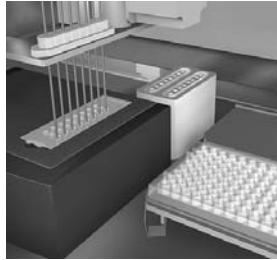


- 3 The Digester destains and equilibrates the gel plugs remaining (prior to enzyme addition and digestion) by one, or several washing procedure(s).
- 4 The robot moves the finished microplate to the dryer for dehydration of the gel plugs under a predetermined time (to enhance enzyme entry into the gel plug before digestion).

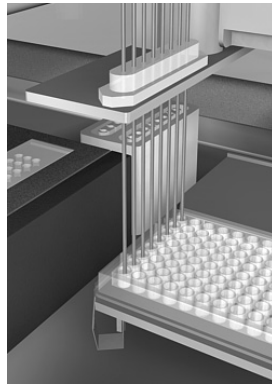


- 5 The robot moves the microplate back to the Digester.

- 6 The multi-probe head moves to the enzyme container.



- 7 The Digester adds enzyme (e.g. Trypsin) which enters into and rehydrates the gel plugs.



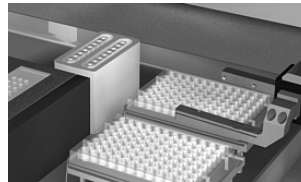
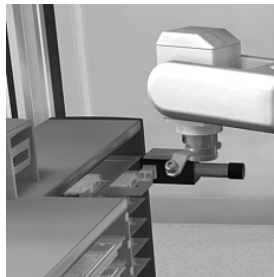
- 8 The Digester adds buffer (if needed).
- 9 The multiple probe head moves to the rinse station.

2 System description

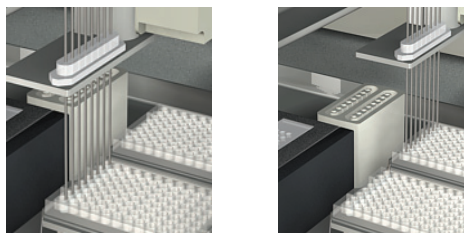
- 10 The robot moves the finished microplate to the incubator for enzymatic digestion under a predetermined time. During incubation, the microplate is covered with a lid to avoid evaporation.



- 11 The robot moves the microplate from the incubator back to the Digester (position in front of the enzyme container).
- 12 The robot moves a new, empty microplate from the hotel to the Digester (position in front of the rinse station).

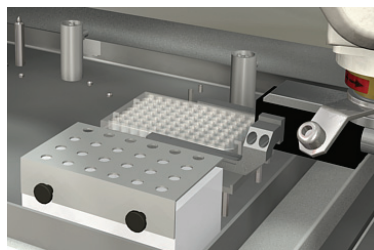


- 13 The Digester moves the digested samples (extracted from the gel) to the empty microplate.

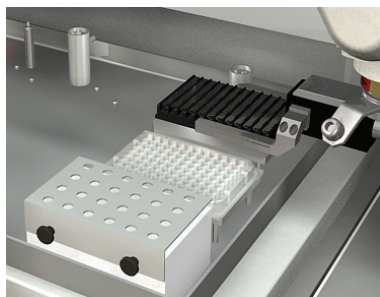


- 14 The robot moves the finished microplate to the dryer for drying under a predetermined time.

- 15 The robot moves the finished microplate to the Spot Picker & Spotter.

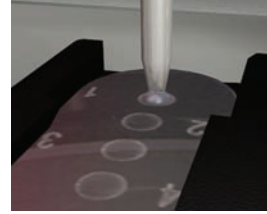
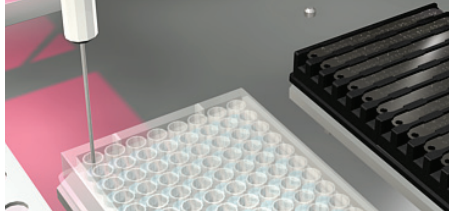


- 16 The robot moves an MS sample tray from the hotel to the position beside the microplate.



2.7.4 Spotting (standard method)

- 1 The Spotter dissolves the sample in the microplate by using Matrix solution.
- 2 The Spotter mixes the sample in the microplate well by a defined number of aspirating/dispensing cycles.
- 3 The Spotter transfers the sample to the MALDI target.



- 4 The sample is left on the MALDI target for air drying.
- 5 The spotter needle moves to the rinse station for rinsing in spotter liquid.



The workstation continues the processing to complete the required batch run.

3 Start-up and shutdown

This chapter provides start-up and shutdown instructions for Ettan Spot Handling Workstation. Refer to *Ettan Spot Handling Workstation User Manual* for instructions on the consumables needed for a batch run.

3.1 Starting the workstation

To start Ettan Spot Handling Workstation:

- 1 Turn on mains power to Ettan Spot Handling Workstation with the mains power switch at the right side of the cabinet, see Fig. 2-6.
- 2 Check that Ettan Spot Handling Workstation is operating:
 - the **Power** indicator should light
 - the PC should show the log-on screen

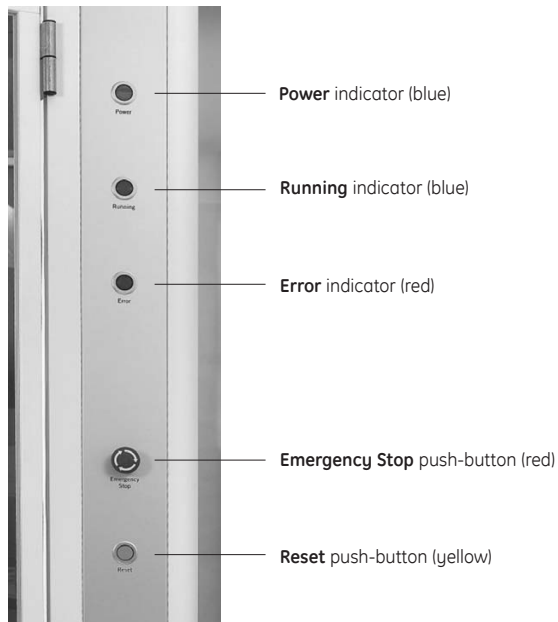


Fig 3-1. Ettan Spot Handling Workstation front panel.

- 3 Log-in to Windows 2000.

Further operating instructions are provided in *Ettan Spot Handling Workstation User Manual*.

3.2 Before batch start-up

Before starting a batch run, the following checks should be done:

CAUTION! Always make sure that no foreign objects are left inside the cabinet.

- 1 Check that the correct spot picker head for the actual batch run is selected. For instructions on changing spot picker head, see *Chapter 4 User Maintenance* of this manual.
- 2 Check that there is not any calibration plate in the Spot Picker & Spotter and the Digester.
- 3 Check that there is not any microplate in the Spot Picker & Spotter, the Digester, the incubator and the dryer.
- 4 Check that there is not any gel tray glass lid in the lower part of the gel hotel.
- 5 Check that all microplates and MS sample trays are placed into the center position in the frame of the hotel shelf.
- 6 Check that tubing and couplings are connected and tightened.
- 7 Check that the waste bottles are empty.

Further operating instructions are provided in *Ettan Spot Handling Workstation User Manual*.

3.3 Pause batch mode

The pause batch mode can be used when a temporary stop is needed, e.g. to remove a foreign object. Proceed as follows:

- 1 In the **Batch Processing** dialog, click the **Pause** button.
- 2 Wait until the **Running** indicator on the cabinet's front panel flashes.
- 3 Open the cabinet door(s) and remove the foreign object.
- 4 Close the cabinet door(s).
- 5 Press the **Reset** button on the cabinet's front panel.

Further operating instructions are provided in *Ettan Spot Handling Workstation User Manual*.

3.4 Shutting down the workstation

When not in use, shut down the workstation:

- 1 In the menubar, choose command **File:Exit**.
- 2 Log-out from Windows 2000.
- 3 Switch off mains power with the mains power switch at the right side of the cabinet.

3.5 Emergency shutdown

In the event of an emergency, press the **Emergency Stop** button on the front panel of Ettan Spot Handling Workstation, see Fig. 3-2 below. When pressing the **Emergency Stop** button or if a cabinet door is opened during a run, the workstation's instruments and the transfer robot immediately cease movement and all motors are de-energized.



WARNING! If the Picker/Spotter or the Digester is in its homing procedure when the emergency stop button is activated, the homing will be completed before the instrument ceases movement. Homing means that the unit is seeking its zero position. The text "HOMING" is shown in the unit's display.



Fig 3-2. The Emergency Stop button on the cabinet's front panel.

3.5.1 Reset the Emergency Stop

IMPORTANT! Do not switch-off mains power. When mains power is switched-on after a switch-off, the transfer robot cannot perform homing. Call GE Healthcare Service.

For information how to reset the emergency stop, re-start or abort a batch run, refer to *Ettan Spot Handling Workstation User Manual*.

4 User Maintenance

User maintenance is important for safe and trouble-free operation of Ettan Spot Handling Workstation. Preventive maintenance should be performed on a yearly basis by qualified service personnel.

This chapter provides instructions for user maintenance and service operations.



WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.



WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and preventive maintenance should be performed by qualified personnel.

Contact your GE Healthcare representative for more service information.

4.1 *System administration*

A competent person at the laboratory should be trained and appointed as system administrator for Ettan Spot Handling Workstation. The system administrator should be responsible for the results and data backup.

4.2 User interface

Parts of the user maintenance are handled from the System Configuration window, see Fig. 4-1 below.

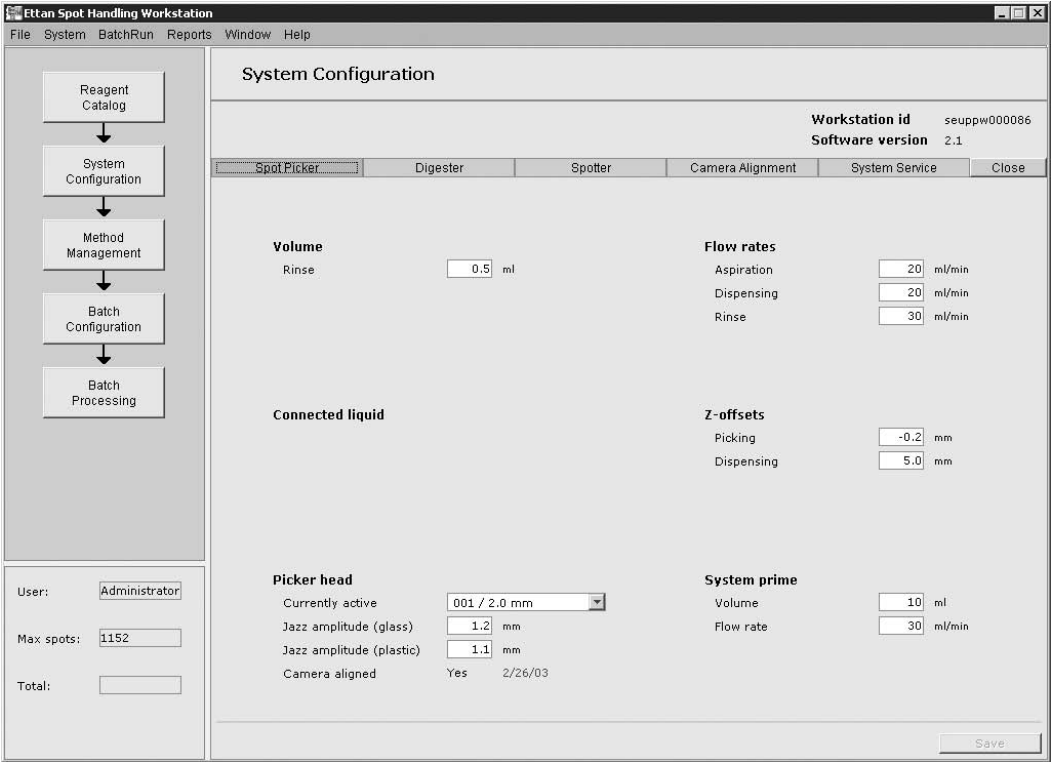


Fig 4-1. The System Configuration window.

4.3 User maintenance schedule

Table 4-1 lists the maintenance operations that should be performed by the user at regular intervals.

Table 4-1. User maintenance.

Interval	Action	Instructions/reference
Daily	Check the transfer robot's working area	Due to the risk of collision, check that no foreign objects are left in the cabinet
When required	Clean the workstation	Wipe the cabinet and the instrument covers with a soft damp tissue. Let dry completely before use. See Section 4.4
	Clean the microplate lids	See Section 4.4.3

4.4 *Cleaning procedures*

4.4.1 Instruments

Cleaning the instruments regularly

For proper function, the instruments should be kept clean and dry. Chemical stains and dust should be removed.

- 1 Wipe the instrument covers with a soft damp tissue. If needed, use a mild detergent to remove stains.
- 2 Let the instrument dry completely before use.
- 3 Rinse the buffer lines with Milli-Q water or equivalent, using the prime syringe tool.

4.4.2 Gel trays

When a batch run is completed:

- 1 Remove the gel from the gel tray and store/discard as appropriate.
- 2 If the gel is to be discarded, scrape the gel off the glass plate with a plastic spacer or similar object. Then put the plates into a 5% Decon™ solution (or equivalent) overnight to remove any gel fragments which adhere to the plate.
- 3 Rinse the liquid out of the gel tray with Milli-Q water or equivalent and leave the gel tray to dry.

4.4.3 Microplate lids

- 1 Wipe the lid with a soft damp tissue. If needed, use a mild detergent.
- 2 Rinse the lid with Milli-Q water or equivalent and let dry.
- 3 Carefully insert the lids into the incubator.

4.5 Camera alignment



WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.

4.5.1 Required accessories

Following accessories are needed for the camera alignment:

- Gel tray
- Camera alignment foot, code no. 18-1143-33
- White paper
- Double-sided adhesive tape

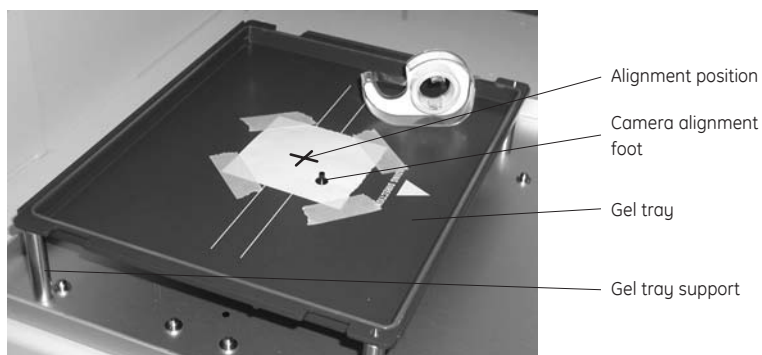


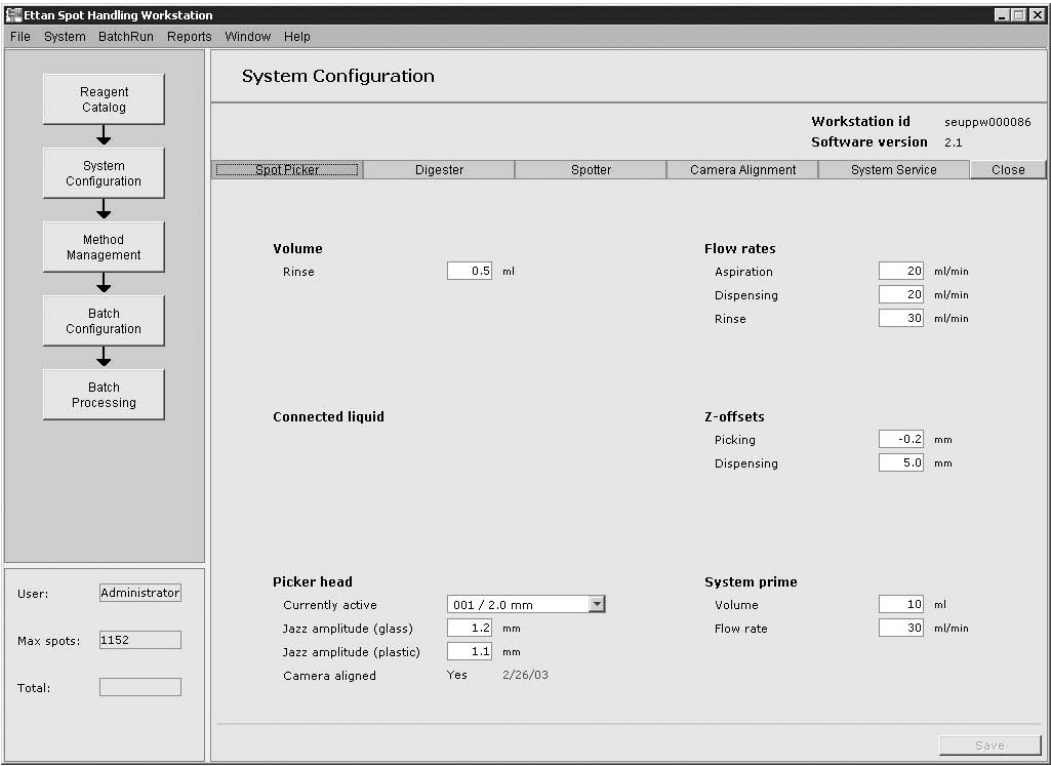
Fig 4-2. Accessories for camera alignment.

4.5.2 Focus and aperture adjustment

Before starting the camera alignment, check that the camera lens is focused on the bottom of the gel tray:

4 User Maintenance

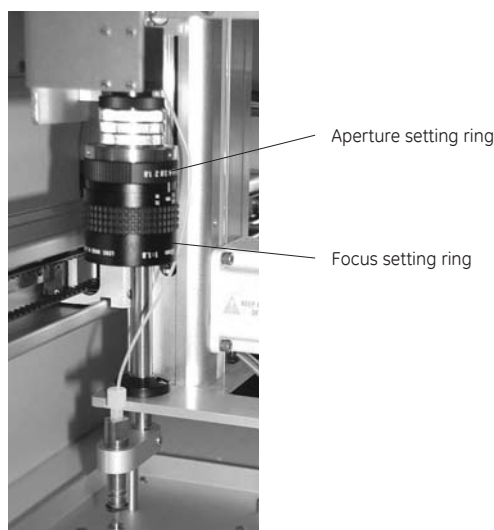
1 From the menubar, select **System:System Configuration**.



- 2 Click on **System Service**.
- 3 Click on **Start**. The Service Utilities dialog appears.



- 4 Click on **View Camera Image**. This will open a camera window on the screen.
- 5 Place the gel tray onto its support in the Picker/Spotter.
- 6 Place a piece of white paper (size approx. 100 × 100 mm) with printed black text into the corner of the tray beneath the camera. Ensure that the paper lays flat on the bottom of the tray.
- 7 If necessary, adjust the aperture to normal brightness by using the aperture setting ring, see figure below.



- 8 Adjust the focus to a sharp picture by using the focus setting ring, see figure above.
- 9 **IMPORTANT!** Set the aperture to **4** (four).
- 10 Remove the gel tray.
- 11 Perform the camera alignment procedure, see Section 4.5.3 below.

4.5.3 Alignment procedure

CAUTION! Before starting camera alignment, make sure that the Zarm is properly mounted as high as possible on the Y-arm.

- 1 Prepare the gel tray as follows, see Fig. 4-2:
 - Place a piece of white paper in the center of the gel tray using adhesive tape.

4 User Maintenance

- Place a strip of double-sided adhesive tape onto the white paper across the alignment position in the center of the gel tray.
 - Place the gel tray onto the gel tray support in the Picker/Spotter.
- 2 From the menubar, select **System: System Configuration**.

Ettan Spot Handling Workstation

File System BatchRun Reports Window Help

Reagent Catalog

System Configuration

Method Management

Batch Configuration

Batch Processing

User: Administrator

Max spots: 1152

Total:

System Configuration

Workstation id: seuppw000086
Software version: 2.1

Spot Picker | Digester | Spotter | Camera Alignment | System Service | Close

Volume

Rinse: 0.5 ml

Flow rates

Aspiration: 20 ml/min
Dispensing: 20 ml/min
Rinse: 30 ml/min

Connected liquid

Z-offsets

Picking: -0.2 mm
Dispensing: 5.0 mm

Picker head

Currently active: 001 / 2.0 mm
Jazz amplitude (glass): 1.2 mm
Jazz amplitude (plastic): 1.1 mm
Camera aligned: Yes 2/26/03

System prime

Volume: 10 ml
Flow rate: 30 ml/min

Save

- 3 Click on the **Camera Alignment** tab.



WARNING! MOVING PARTS. Do not enter the Spot Picker & Spotter's working area directly after clicking the camera alignment tab since the picker/spotter head and camera assembly can make sudden, rapid movements. Wait at least 30 seconds before applying the camera alignment foot.

- 4 Apply the calibration alignment foot onto the picker head.



- 5 Close the cabinet door.
- 6 Press **Reset** on the cabinet's front panel.
- 7 Check the **Confirm that the marker is placed on the pick head box**.

File System BatchRun Reports Window Help

System Configuration

Workstation id seuppw000086
Software version 2.1

Spot Picker	Digester	Spotter	Camera Alignment	System Service	Close								
Place the marker on the picker head and press start to start the alignment iteration.													
Alignment status Not aligned													
<table border="1"> <thead> <tr> <th>Iterations</th> <th>1</th> <th>2</th> <th>3</th> </tr> </thead> <tbody> <tr> <td>Status</td> <td>Not performed</td> <td>Not performed</td> <td>Not performed</td> </tr> </tbody> </table>						Iterations	1	2	3	Status	Not performed	Not performed	Not performed
Iterations	1	2	3										
Status	Not performed	Not performed	Not performed										

User: Administrator

Max spots: 1152

Total:

☐ Confirm that the marker is placed on the picker head

Start Save

8 Click the **Start** button.



WARNING! MOVING PARTS. Do not enter the Spot Picker & Spotter’s working area directly after clicking the **Start** button since the picker/spotter head and camera assembly can make sudden, rapid movements.

The status bar is yellow during processing and turns into green when the camera alignment is completed.

Workstation idFS.2
Software version2.1

Spot Picker

Digester

Spotter

Camera Alignment

System Service

Close

Place the marker on the pick head and press start to start the alignment iteration.

Alignment status

Not aligned

Iterations

1

2

3

Status

Complete

Not performed

Not performed

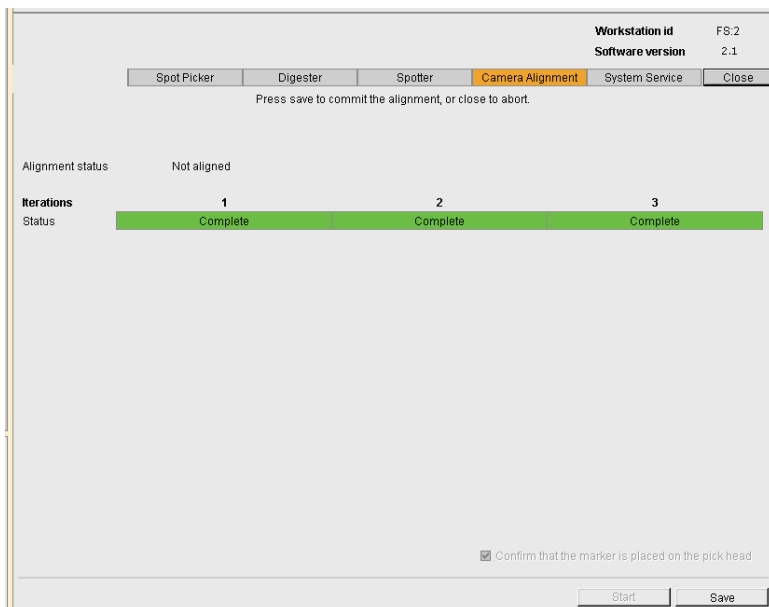
☐ Confirm that the marker is placed on the pick head

Start

Save

9 Open the cabinet door.

10 Repeat steps 4 to 8 until all status bars are green.



11 Click the **Save** button to save the alignment values.

12 Remove the gel tray.

13 Close the cabinet door.

14 Press **Reset** on the cabinet's front panel.

4.6 Changing the spotter needle



WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.

Note: Calibration plate, code no. 18-1156-66, is needed for the changing procedure.

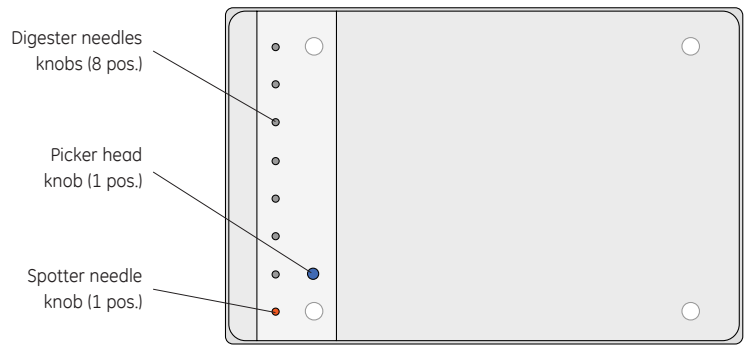


Fig 4-3. Calibration plate 18-1156-66.

4.6.1 Changing procedure

- 1 From the menubar, select **System:System Configuration**.

Ettan Spot Handling Workstation

File System BatchRun Reports Window Help

System Configuration

Workstation id: seuppw000086
Software version: 2.1

Spot Picker Digester Spotter Camera Alignment System Service Close

Volume
Rinse: 0.5 ml

Flow rates
Aspiration: 20 ml/min
Dispensing: 20 ml/min
Rinse: 30 ml/min

Connected liquid

Z-offsets
Picking: -0.2 mm
Dispensing: 5.0 mm

Picker head
Currently active: 001 / 2.0 mm
Jazz amplitude (glass): 1.2 mm
Jazz amplitude (plastic): 1.1 mm
Camera aligned: Yes 2/26/03

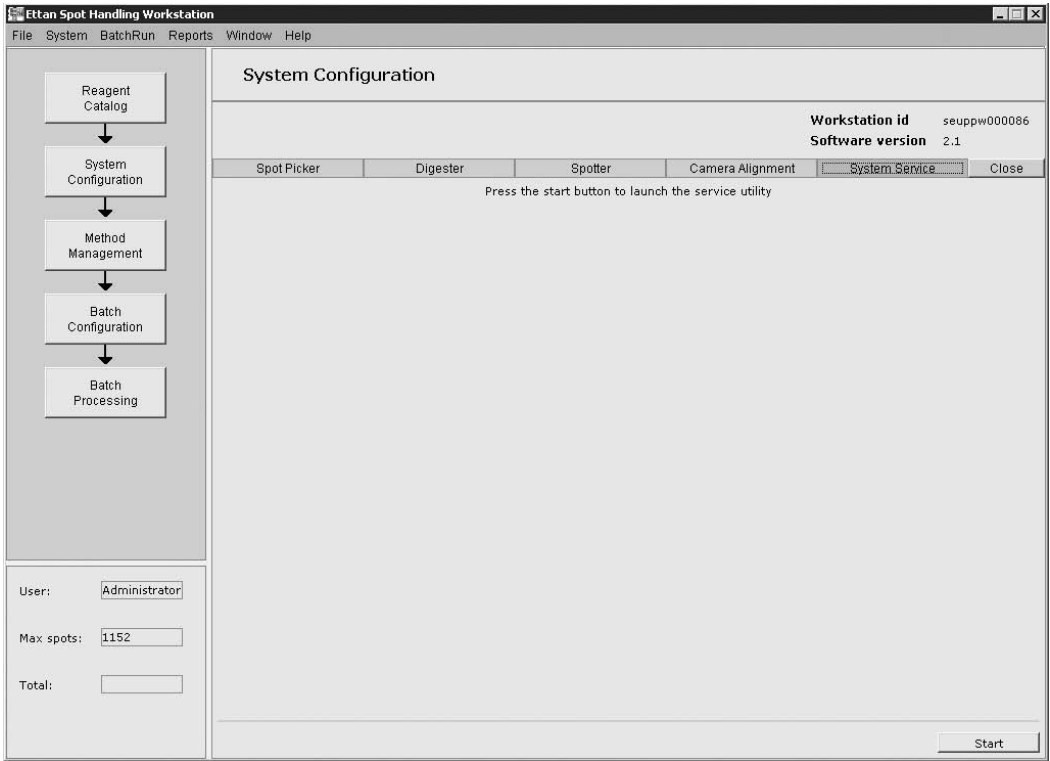
System prime
Volume: 10 ml
Flow rate: 30 ml/min

User: Administrator
Max spots: 1152
Total:

Save

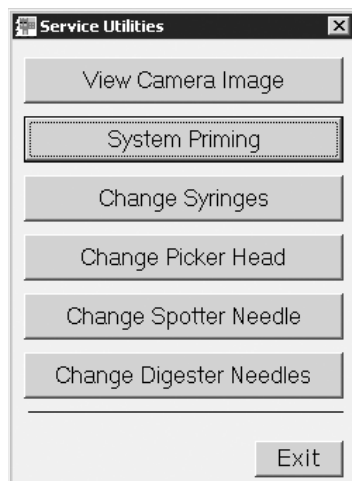
4 User Maintenance

2 Click on **System Service**.



3 Click on **Start**.

The Service Utilities dialog appears.

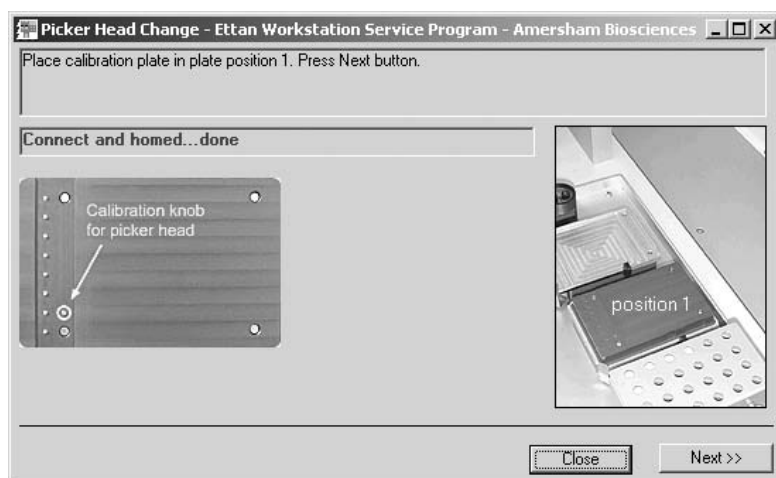


- 4 In the Service Utilities dialog, click on **Change Spotter Needle**.
- 5 Open the cabinet door.

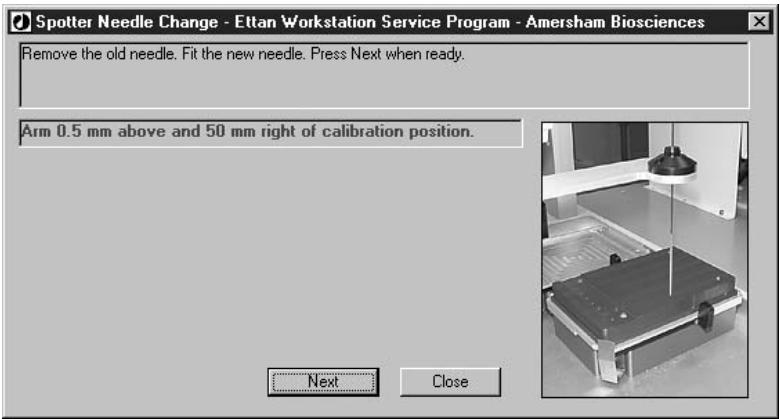


WARNING! MOVING PARTS. Do not enter the Spot Picker & Spotter's working area directly after opening the cabinet door(s) since the picker/spotter head can make sudden, rapid movements. Wait at least 30 seconds before entering this working area.

- 6 Place the calibration plate onto plate position 1.



7 In the Spotter Needle Change dialog, click on **Next**.

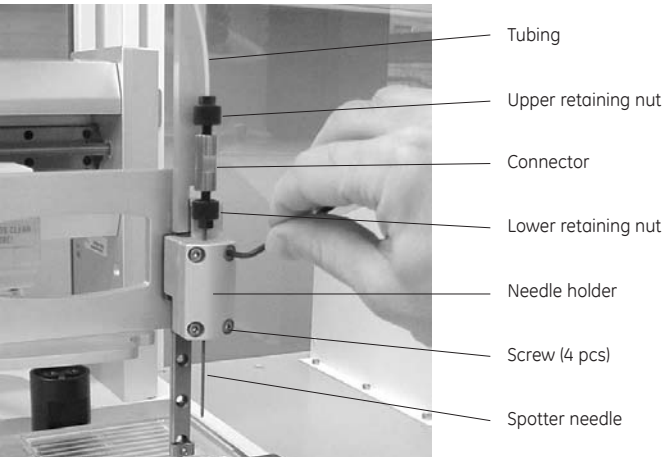


The spotter needle moves to a position 1.0 mm above and 50 mm to the right of the calibration position.

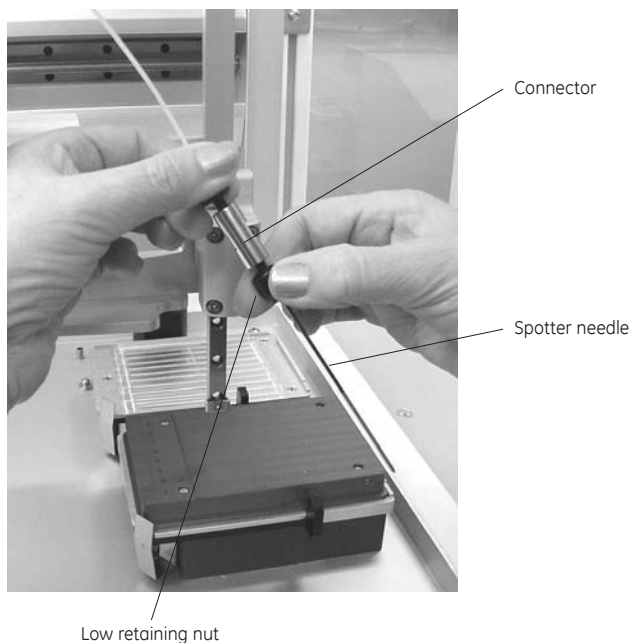
Note: The Spotter Needle Change dialog shows a picture of the previous spotter head design.

8 Replace the spotter needle as follows:

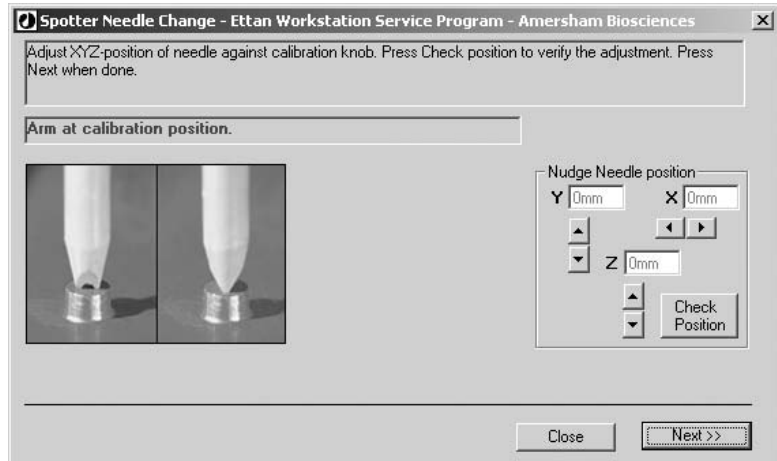
- Loosen the four needle holder screws with an Allen key, see figure below. Do not remove the needle holder.



- Lift out the needle with its tubing and connector from the needle holder, see figure below.



- Loosen the lower retaining nut. Use a wrench to hold the connector while loosen the nut. Do not remove the retaining nut.
- Remove the worn needle.
- Carefully insert the new needle. Press the needle against its innermost position.
- Fingertighten the retaining nut.
- Carefully insert the needle with its tubing and connector into the needle holder.
- Tighten the four screws while holding the tip of the needle approx. 1 mm above the calibration plate. Be carefully not to scratch the needle against the calibration plate.

9 Click on **Next**.

The spotter needle moves to the calibration position.

10 Adjust the **XYZ-positions** against the calibration plate using the **Nudge Needle position** buttons as follows:

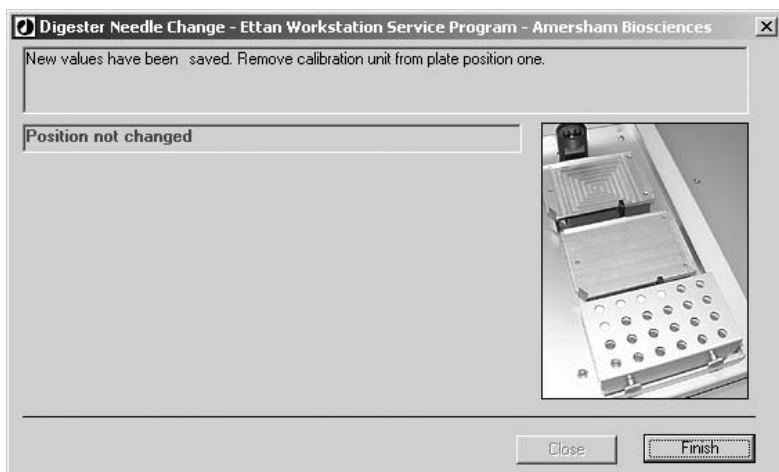
- Carefully lower the needle towards the calibration knob. Stop lowering when the needle is approx. 0.5 mm above the calibration knob.
- Adjust the **XY-position** against the center of the calibration knob. The tip of the needle must point precisely to the center of the calibration knob.

Press the **Check Position** button to verify the adjustment. The picker/spotter head moves to the home position and then back to its new XY-position. If necessary, re-calibrate the XY-position.

- Adjust the **Z-position** so that a strip of thin aluminum foil is barely stuck between the needle and the knob. Do as follows:
 1. Carefully lower the needle (0.1 mm steps) while moving slightly the aluminum foil sideways. Stop lowering when the foil is stuck.
 2. Press the **Check Position** button to verify the adjustment. The foil should stick.
 3. Raise the needle 0.1 mm and press the **Check Position** button to verify. The aluminum foil should not stick.
 4. Lower the needle 0.1 mm and press the **Check Position** button to verify that the aluminum foil now sticks. Re-calibrate if necessary.

- Check that the tip of the needle points precisely to the center of the calibration knob, see figure above (item 4.6.1.9).
- If necessary, re-calibrate the XY-position.

11 Click on **Next**.



12 Click on **Finish** to save the changed position values.

13 Remove the calibration plate.

4.7 Changing the digester needles

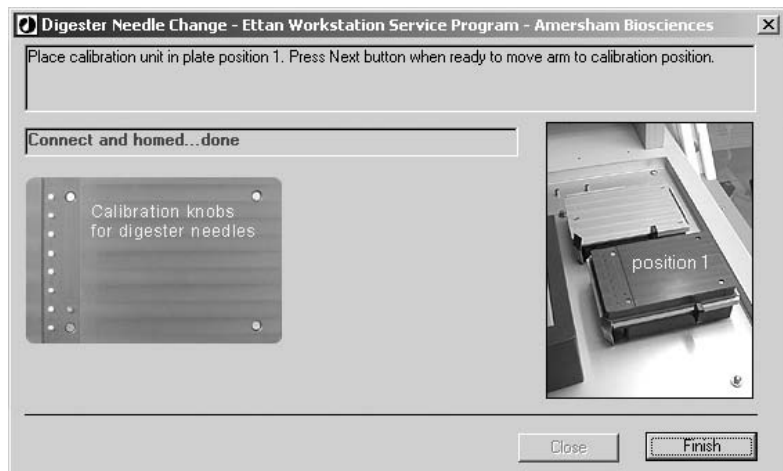


WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.

Note: Calibration plate, code no. 18-1156-66, is needed for the changing procedure, see Fig. 4-3.

4.7.1 Changing procedure

- 1 From the menubar, select **System: System Configuration**.
- 2 Click on **System Service**, then click **Start**.
- 3 In the Service Utilities dialog, click on **Change Digester Needles**.



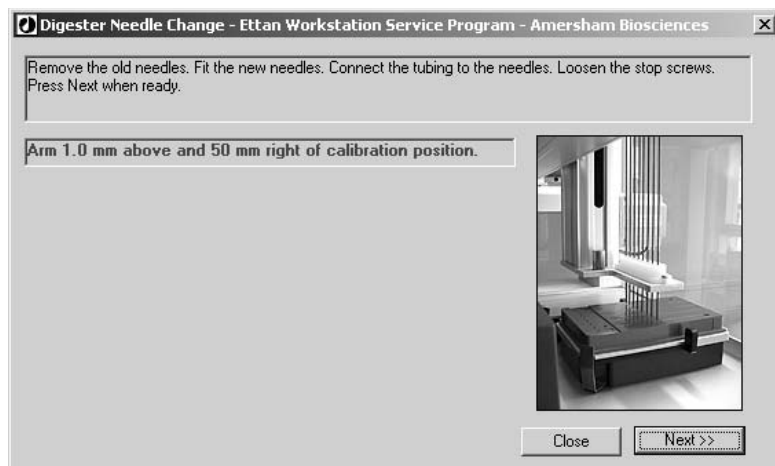
- 4 Open the cabinet door.



WARNING! MOVING PARTS. Do not enter the Digester's working area directly after opening the cabinet door(s) since the digester head can make sudden, rapid movements. Wait at least 30 seconds before entering this working area.

- 5 Place the calibration plate onto plate position 1.

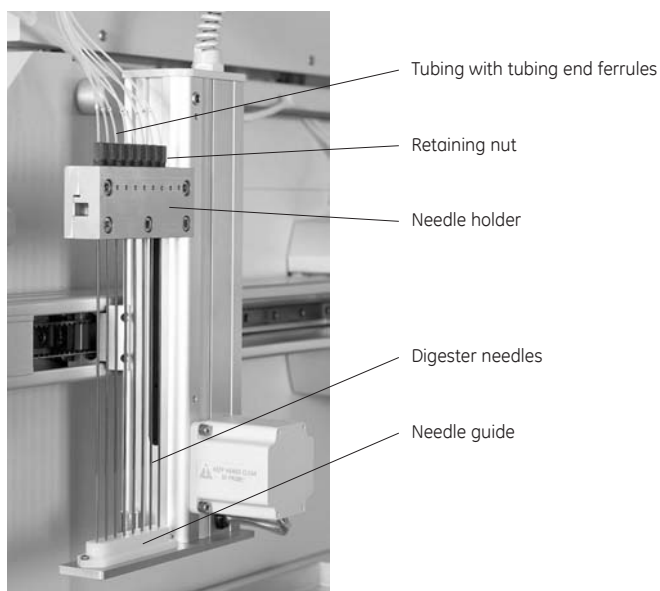
- 6 In the Digester Needle Change dialog, click on **Next**.



The digester needles move to a position 1.0 mm above and 50 mm to the right of the calibration position.

- 7 Replace the digester needles as follows:

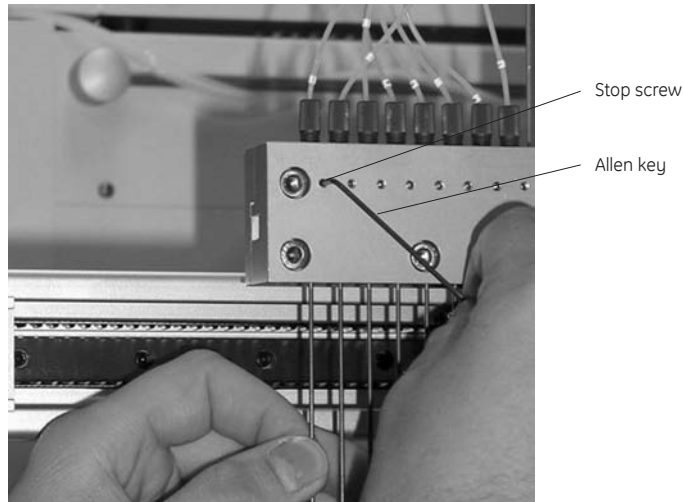
- Loosen and remove the retaining nuts with its tubing on top of the needle holder



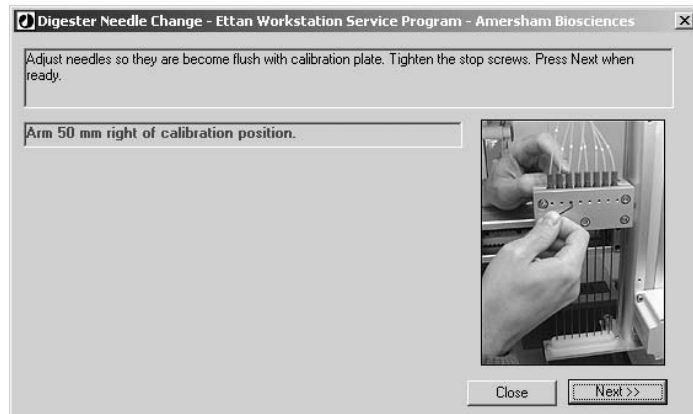
Note: Be careful not to lose the tubing end ferrules.

- Remove the worn needles

- Carefully insert the new needles
- Let the needles drop down through the holes in the needle guide
- Attach the retaining nuts with its tubing
- Finger tighten the retaining nuts
- Loosen the stop screw with an Allen key

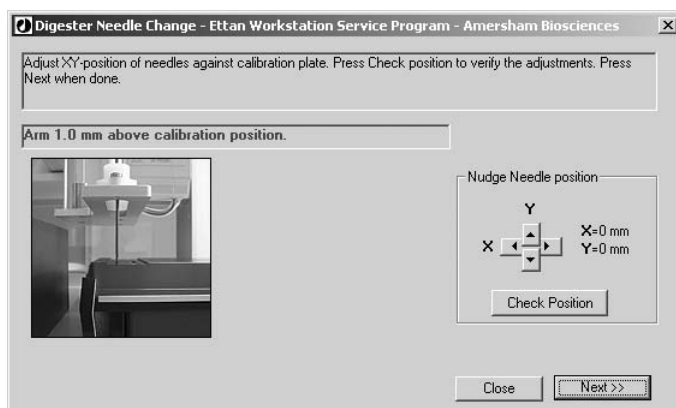


8 Click on **Next**.



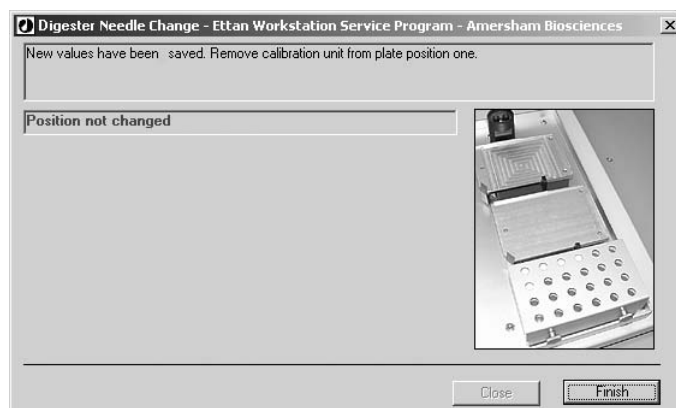
- 9 Check that the needles are flush with the calibration plate, i.e. the needles should be resting on the calibration plate. Adjust if necessary.
- 10 Tighten the stop screws while holding the needles slightly against the calibration plate.

- 11 Check that the needles are flush with the calibration plate. If not, repeat the procedure.
- 12 Click on **Next**.



The digester needles move to a position 1.0 mm above the calibration position.

- 13 Adjust the XY-position of the needles against the calibration plate using the **Nudge Needle position** buttons. The tip of the needles must be clearly within the area of the calibration knobs.
- 14 Press the **Check Position** button to verify the adjustments. The digester needles move to the home position and then back to the new XY-position.
- 15 Visually check the needle positions. If necessary, re-calibrate XY-positions.
- 16 Click on **Next**.



4 User Maintenance

- 17 Click on **Finish** to save the changed position.
- 18 Remove the calibration plate.

4.8 Changing picker head



WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.

The Spot Picker can be used with two sizes of picker heads (1.4 and 2.0 mm). The picker heads are easily changed by a simple screw-in/screw-out method. After changing a picker head, a camera alignment should be performed to establish the offset between the picker head and the camera lens. The gel Z-position may also have to be adjusted in the System Configuration Spot Picker window.

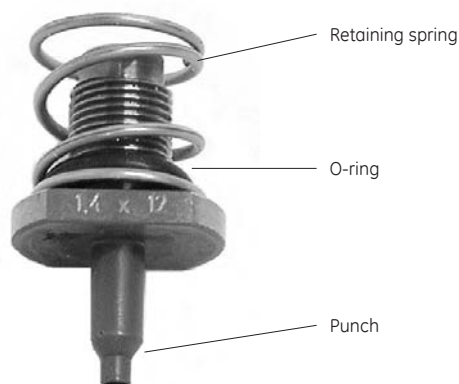


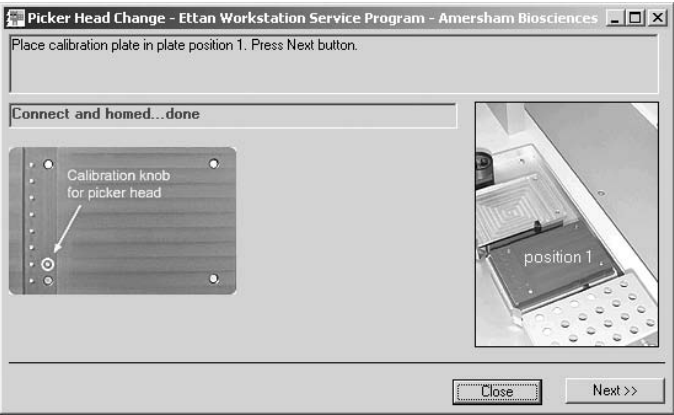
Fig 4-4. The picker head.

Note: Calibration plate, code no. 18-1156-66, is needed for the changing procedure, see Fig. 4-3.

4.8.1 Changing procedure

- 1 From the menubar, select **System: System Configuration**.
- 2 Click on **System Service**, then click **Start**.

3 In the Service Utilities dialog, click on **Change Picker Head**.



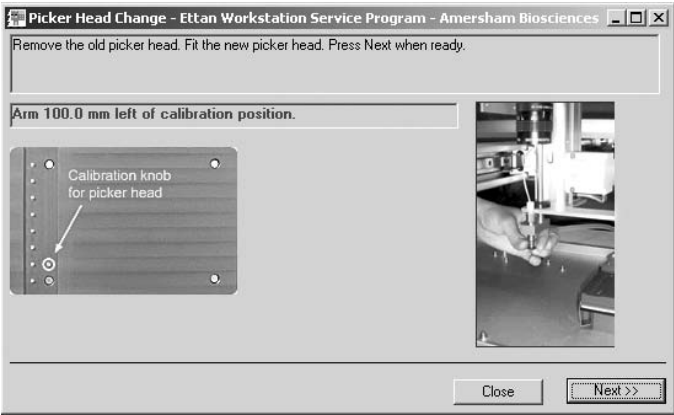
4 Open the cabinet door.



WARNING! MOVING PARTS. Do not enter the Spot Picker & Spotter's working area directly after opening the cabinet door(s) since the picker/spotter head can make sudden, rapid movements. Wait at least 30 seconds before entering this working area.

5 Place the calibration plate onto plate position 1.

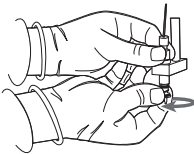
6 In the Picker Head Change dialog, click on **Next**.



The picker head moves to a position 100.0 mm to the left of the calibration position.

7 Remove the picker head as follows:

- Hold the top of the picking piston with one hand, and with the other unscrew the picker head from the base of the piston.



- When the picker head is completely unscrewed from the base of the piston, place it in a safe location until it is required once more. Make sure that the retaining spring is also kept safe.
- 8 Attach the new picker head as follows:
- Place the retaining spring over the screw thread on the top of the picker head.
 - Screw the picker head onto the base of the piston, making sure that the screw thread is inside the piston. At the same time, hold the top of the piston steady with your other hand.
 - Slowly screw the picker head into the piston while holding the top of the piston still with the other hand. Tighten the picker head against the stop position.

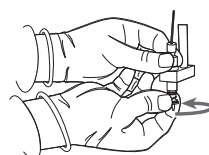
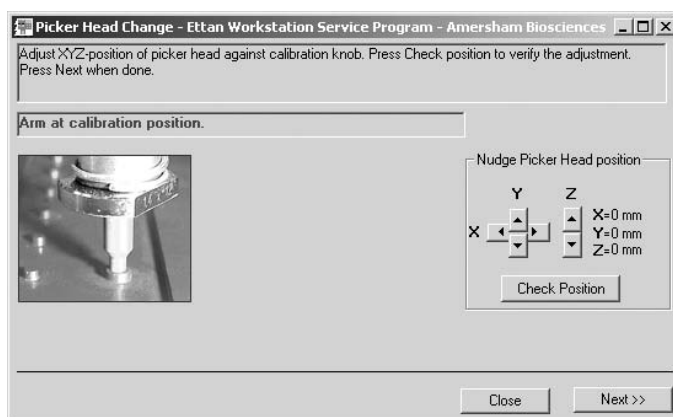


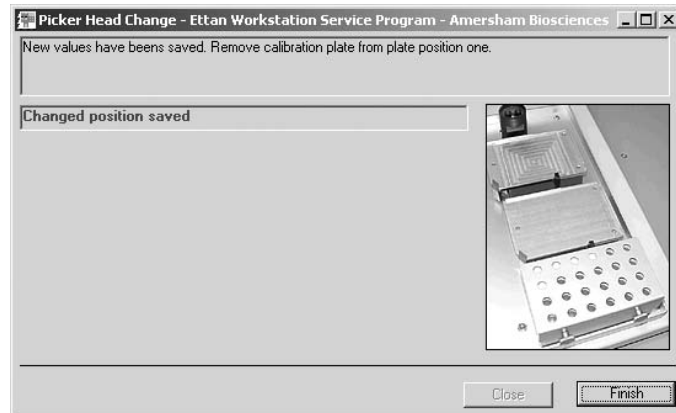
Fig 4-5. Final appearance of the assembled picker head.

- 9 In the Picker Head Change dialog, click on **Next**



The picker head moves to the calibration position.

- 10 Adjust the XYZ-position of the picker head against the calibration knob using the **Nudge Picker Head position** buttons.
- 11 Press the **Check Position** button to verify the adjustment. The picker head moves to the home position and then back to its new XYZposition.
- 12 Click on **Next**.



- 13 Click on **Finish** to save the changed position.
- 14 Remove the calibration plate.

Proceed by calibrating the distance between the picker head and the camera. For instructions on camera alignment, see Section 4.5.

IMPORTANT! The new picker head must be registered in the "Picker Head Management", see *Ettan Spot Handling Workstation User Manual*.

4.9 Priming

Priming the syringes removes air bubbles in the spot picker/spotter and digester tubing, and also rinses the tubing with fresh solution. Priming is performed as follows:

- before starting spot picking, spotting and digestion processing, see descriptions 4.9.1, 4.9.2, 4.9.3 and 4.9.4 below
- automatically during spotting and digestion processing However, additional priming should be performed:
- after replacement of syringes
- if the system will not be used for a longer period of time, and is to be filled with distilled water
- if the system is contaminated

Note: After priming, check that all tubing, outside and inside the workstation, are filled with liquid and no air is visible.

Note: Priming can also be done from the Batch Processing window, refer to Ettan Spot Handling Workstation User Manual.

4.9.1 Priming the Digester

- 1 Check that the tube fittings are properly tightened.
- 2 From the menubar, select **System:System Configuration**.
- 3 Click on **System Service**, then click **Start**.
- 4 In the Service Utilities dialog, click on **System Priming**.

System Priming - Ettan Workstation Service Program - Amersham Biosciences

Estimated Duration: 0h 7min

Priming is not started

Start

Priming of digester is enabled

Priming of picker is enabled

Priming of spotter is enabled

Abort

Exit

Digester | Picker | Spotter

☒ Enable Digester Priming

Valve Positions to be primed:

Reservoir to valve priming volume (0 - 50): 5 ml

Valve to needle priming volume (0 - 50): 5 ml

flow rate (1 - 16.87): 16.87 ml/min

Valve Position 1 ☒ Valve Position 5 ☒

Valve Position 2 ☒ Valve Position 6 ☐

Valve Position 3 ☒ Valve Position 7 ☐

Valve Position 4 ☒ Valve Position 8 ☐

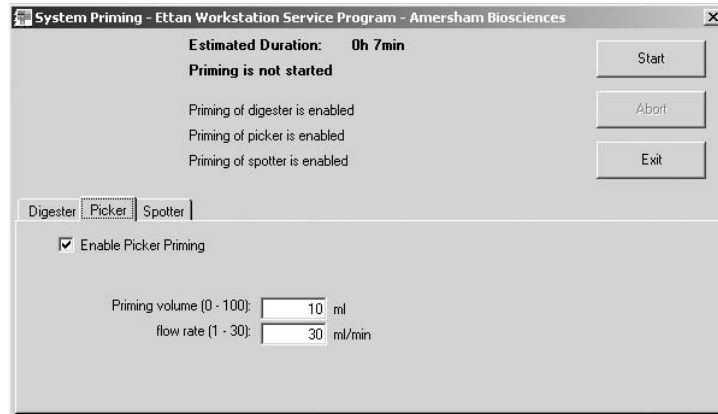
Valve Position for valve to needle priming: 5

The System Priming Digester dialog appears.

- 5 Check the **Enable Digester Priming** box.
- 6 Enter recommended values and check the **Valve Position** boxes for the valve positions which have connected liquids.
- 7 Click on **Start**.
- 8 The Digester moves to its rinse station.
- 9 When priming is finished, click on **Exit**.

4.9.2 Priming the Picker

- 1 Check that the tube fittings are properly tightened.
- 2 In the System Priming dialog, select **Picker**.

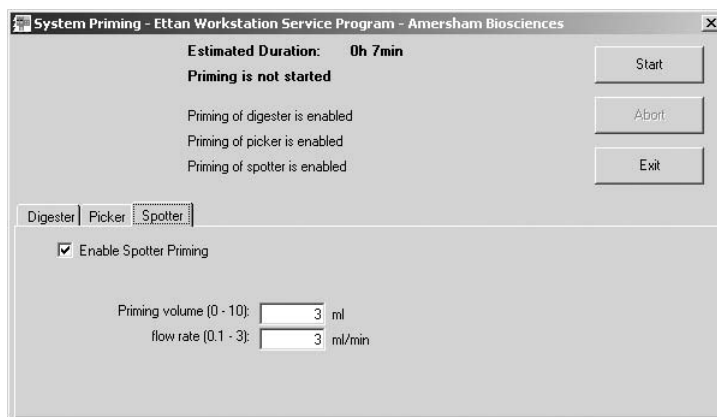


- 3 Check the **Enable Picker Priming** box.
- 4 Enter recommended values.
- 5 Click on **Start**.
- 6 The Spot Picker & Spotter moves to its rinse station.
- 7 When priming is finished, click on **Exit**.

4.9.3 Priming the Spotter

- 1 Check that the tube fittings are properly tightened.

- 2 In the System Priming dialog, select **Spotter**.



- 3 Check the **Enable Spotter Priming** box.
- 4 Enter recommended values.
- 5 Click on **Start**.
- 6 The Spot Picker & Spotter moves to its rinse station.
- 7 When priming is finished, click on **Exit**.

4.9.4 System priming

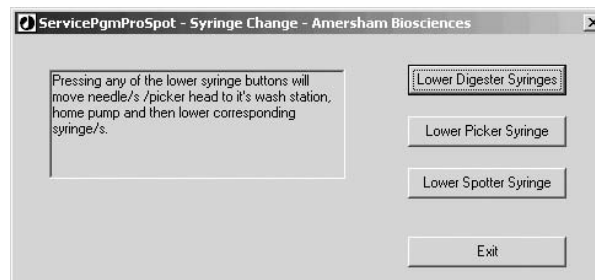
Priming of the Digester, the Picker and the Spotter can also be done at the same time. Do as follows:

- 1 Check the **Enable Digester Priming**, the **Enable Picker Priming** and the **Enable Spotter Priming** boxes.
- 2 Enter recommended values.
- 3 Click on **Start**.
- 4 When priming is finished, click on **Exit**.

4.10 Changing syringes

WARNING! MOVING PARTS. Do not read any barcodes with the external barcode reader while performing user maintenance inside the cabinet. Output signals from the barcode reader will be interpreted by the Spot Picker & Spotter and Digester units as a command to move to their home positions.

- 1 From the menubar, select **System:System Configuration**.
- 2 Click on **System Service**, then click **Start**.
- 3 In the Service Utilities dialog, click on **Change Syringes**.



- 4 To enable replacement of syringe(s), click on **Lower Digester Syringes**, **Lower Picker Syringe** or **Lower Spotter Syringe** respectively. The following will happen:

- The needle/needles/picker head move(s) to its rinse station
- The syringe pump moves to its home position
- The syringe(s) lowers/lower

Note: When replacing the spotter syringe, remember to mount the enclosed sealing into the connection between the tubing and the new syringe.

- 5 Before replacement, the plunger of the new syringe should be wetted as

follows:

- Remove the plunger from the barrel
 - Dip the plunger tip into Milli-Q water, and re-insert the plunger into the glass barrel
 - Work the plunger up and down 5-6 times, re-wetting the tip, if necessary
- 6 After replacement of syringe(s), click on **Exit**.

4.11 *Preventive maintenance*

GE Healthcare recommends preventive maintenance (PM) to be performed by qualified service personnel during the yearly service visit. The extent of the PM is to be agreed in the service contract.

Recycling



This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

5 Troubleshooting

For information on troubleshooting, refer to *Ettan Spot Handling Workstation User Manual* and *Online Help*.



Recycling

This symbol indicates that the waste of electrical and electronic equipment must not be disposed as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.

6 Specifications

For complete specifications of each component, refer to the individual hardware documentation.

Relevant system specifications are listed below.

6.1 *Technical specifications*

Table 6-1. Ettan Spot Handling Workstation technical specifications.

Ettan Spot Handling Workstation

Mains voltages	100/120/230/240 V AC + 10-15%, 50/60 Hz \pm 2 Hz
Operating temperature	18–30 °C (65–86 °F)
Dimensions	251.5 × 129 × 198.5 cm (W × D × H)
Total weight	645 kg
Power consumption	1600 VA
Sample source	Polyacrylamide gels cast on glass or GelBond PAG film backing
Maximum gel size	276 × 212 mm
Minimum gel size	180 × 160 mm
Gel thickness compatibility	1 mm
Spot picker head diameter	1.4, 2.0 mm
Detection system compatibility	Silver, Coomassie™, fluorescence
Barcode reader:	
Max. output radiation	1.2 mW (223 us)
Emitted wavelength	670 nm
Max. beam divergence	60 degrees

6.2 Performance specification

Table 6-2. Ettan Spot Handling Workstation performance specification.

Ettan Spot Handling Workstation	
Process capacity	12 Ettan DALT II gels and 1152 protein spots per batch in approx. 30 hours (depending on method).

6.3 Regulatory requirements

Ettan Spot Handling Workstation meets or exceeds the standards listed in Table 6-3.

Table 6-3. Regulatory requirements, Ettan Spot Handling Workstation.

Safety	UL 1260, UL 1492 (camera), CSA C22.2 - No. 151, IEC 1010-1
EMC	EN 50082-1, EN 50022 (frame grabber), FCC Class A
EMI	EN 50081-1, EN 50024 (frame grabber)

6.4 Ordering information

6.4.1 Accessories

Table 6-4 lists the accessories that can be ordered from GE Healthcare.

Table 6-4. Accessories for Ettan Spot Handling Workstation.

Product	Quantity	Code No.
Ettan Spot Handling Workstation:		
Ettan Microplate 96 well, 100 pcs	1	18-1150-42
Reagent bottle	1	18-1158-79
Waste bottle	1	18-1158-81
Sealing for incubator lid, 12 pcs	1	18-1160-40
Gel tray trolley	1	18-1149-79
Spot Picker:		
Picker head 1.4 mm for 1.0 mm thick gels	1	18-1147-03
Picker head 2.0 mm for 1.0 mm thick gels	1	18-1143-64
Reference markers, sheet incl. 560 pcs	2	18-1143-34
Gel holder, 2 pcs	1	18-1156-27
Gel tray incl. gel holders and glass lid	1	18-1150-39
Glass lid for gel tray	1	18-1156-28
Syringe, 1 ml	1	18-1143-41
Tubing and fittings	1	18-1143-63
Inlet tubing package	1	18-1156-29
Camera alignment foot	5	18-1143-33
Spotter:		
Syringe, 100 µl	1	18-1143-55
Needle	1	18-1166-15
Tubing and fittings	1	18-1166-14
Vial with cap, 1 ml, 100 pcs	1	18-1152-75

6 Specifications

Product	Quantity	Code No.
Ettan Sample Slide Kit	2	18-1147-18
MS sample tray:		
Bruker*, Bruker Daltonics	1	18-1155-99
Micromass*, Micromass Ltd.	1	18-1156-00
Voyager™*	1	18-1156-02
Digester:		
Tubing and fittings	1	18-1156-31
Syringes, 500 µl	8	18-1170-86
Needles 2 18-1143-57		
Enzyme container, 48 pcs	1	18-1157-75
Calibration plate	1	18-1156-66

* Delivered without targets.

6.4.2 Related products

Table 6-5 list products that are related to Ettan Spot Handling Workstation. These products can be ordered from GE Healthcare.

Table 6-5. Products related to Ettan Spot Handling Workstation. For code numbers marked with an asterisk (*), see www.gehealthcare.com.

Product	Code No.
Ettan DALT II large vertical gel system	*
Ettan DALT II Pre-cast Gel 12.5	17-6002-36
Ettan DALT II Buffer Kit	17-6002-50
Immobiline™ DryStrip gels and buffers	*
Trypsin, 10 × 250 µg for 10 full batch runs (10 × 1152 samples)	17-6002-75
TFA, 50 ml for 10 full batch runs (10 × 1152 samples)	17-6002-76
4-HCCA, 10 × 20 mg (pre-weighed) for 10 full batch runs (10 × 1152 samples)	17-6002-77
ImageMaster 2D Elite Software	80-6350-56
ImageScanner	18-1134-45
ImageScanner Geometry Calibration Kit	18-1145-55
Typhoon 8600	*
Ettan MALDI-ToF Pro 120 V	18-1156-54
Ettan MALDI-ToF Pro 220 V	18-1156-53

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www.gehealthcare.com

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